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CYTOLOGICAL STUDIES ON DIPNOI, URODELA, ANURA, AND EMYS

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(The publication of this thesis in English has been assisted by the British Council)

INTRODUCTION.

ALTHOUGH in the infancy of chromosome research several epoch-making studies were made on zoological material (McCLUNG, 1905, 1914; BOVERI, 1909; JANSSENS, 1909; AGAR, 1911, 1912), recent advances in cytology have been based, in increasing degree, on botanical material. Nowadays the number of chromosomes and their morphology are known as regards a large number of plant families, and special problems regarding the mechanism and structure of chromosomes have been tackled and solved, with the large chromosomes of certain Monocotyledons as material.

Modern cytological studies on animals are much rarer. There are indeed a large number of studies on the cytology of insects, but our knowledge of the cytological conditions in animals is otherwise extremely scanty. This remark applies particularly to certain groups such as the lower Vertebrata. The number of chromosomes has hitherto been ascertained as regards about 300 species of vertebrates, including about 200 species of Amniota (OGUMA and MAKINO, 1937). As for general cytological problems, merely a few studies have been made on vertebrates, chiefly dealing with the sex chromosomes in Mammalia (KOLLER, 1936, 1937; WHITE, 1940; as regards earlier literature, see OGUMA and MAKINO, 1937).

The object of the investigation reported in the present work was firstly to describe the morphology and number of chromosomes in certain of the lower vertebrates, and secondly to ascertain whether the laws of cellular mechanism previously found in plants and insects are applicable also to these animal organisms. Moreover, certain experiments have been made for the purpose of throwing special light on certain cytological problems, such as the factors determining the chiasma frequency, the influence of temperature upon chiasma frequency, the time when the chromosomes divide, etc. Great importance has also been attached to the possibilities of drawing conclusions from

cytological facts in regard to the phylogenetic connection of the various groups examined.

MATERIALS AND METHODS.

Unfortunately, owing to the war, the number of species had to be confined chiefly to those which could be procured within the country.

The material used for the present study consisted firstly of embryos, secondly of testicles and ovaries from the following species:

Dipnoi.

Epiceratodus Forsteri KREFFT. 5 specimens from Australia. Embryos.
Protopterus annectens OWEN. 5 » » Gambia. »
» » 1 specimen » » Testes.

Urodela.

Ambystoma mexicana COPE. 30 specimens. Derived from animals bought from SCHOLTZE und PÖTSCHKE, Berlin. Embryos.
Pleurodeles waltli MICHAH. 1 specimen. Bought from SCHOLTZE und PÖTSCHKE, Berlin. Testes.
Triturus cristatus LAUR. 20 specimens from Viggbyholm, in the province of Uppland (Sweden). Testes.
» » 5 » » Vollsjö, in the province of Scania (Sweden). Testes.
» » 10 » » Viggbyholm. Embryos.
» » 2 » » » Ovaries.
Triturus vulgaris L. 20 specimens from Viggbyholm. Testes.
» » 10 » » Vollsjö. Testes.
» » 30 » » Viggbyholm. Embryos.
» » 2 » » » Ovaries.
» » *3n.* 1 specimen » Scania. (J. A. BÖÖK's preparations.) Testes.

Anura.

Bufo calamita LAUR. 10 specimens from Charlottenlund, Denmark. Testes.

Bufo calamita LAUR. 5 specimens from Lomma, in the province of Scania. Testes.

Bufo viridis LAUR. 5 » » Charlottenlund, Denmark. Testes.

» » 5 » » Lomma. Testes.

Bufo vulgaris LAUR. 10 » » Viggbyholm. Testes.

» » 5 » » Södertälje, in the province of Södermanland (Sweden). Testes.

» » 5 » » Viggbyholm. Ovaries and ovotestes.

» » 10 » » Hjälstaviken, in the province of Uppland. Embryos.

Discoglossus pictus OTTH. 10 specimens. Derived from animals bought from SCHOLTZE und PÖTSCHKE, Berlin. Embryos.

Hyla arborea L. 5 specimens from Germany. Testes.

Pelobates fuscus LAUR. 4 specimens from the province of Scania. Testes.

» » 10 » » the province of Scania. Embryos.

Rana arvalis NILSS. 10 specimens from Viggbyholm. Testes.

» » 10 » » Järva, in the province of Uppland. Testes.

» » 2 » » Viggbyholm. Ovaries.

» » 20 » » » Embryos.

Rana esculenta L. 4 specimens from Skabersjö, in the province of Scania. Testes.

» » 4 » » Bornholm, Denmark. Testes.

» » *3n.* 4 » » » » »

Rana temporaria L. 10 specimens from Södertälje. Testes.

» » 5 » » Viggbyholm. Testes.

» » 2 » » » Ovaries.

» » 10 » » » Embryos.

Xenopus laevis DAUD. 4 specimens. Derived from animals bought from SCHOLTZE und PÖTSCHKE, Berlin. Testes.

» » » 10 » Same derivation as above. Embryos.

Reptilia.

Emys orbicularis L. 3 specimens. Derivation unknown. Testes.

As a general rule, I have used squash preparations, treated according to the acetocarmin method. Certain modifications in this method were, however, made, partly in collaboration with G. SVÄRDSON. The slides were covered with a film of albumin glycerine, which was then dried over a gas jet. As soon as the slide had cooled, the fixed object was laid on it and crushed between the slide and a greased cover glass. Especially as regards testicle preparations, the pressure on the slide when crushing should be very hard. The fixing proceeded for one minute in 45 % acetic acid and then for two minutes in BELLING's iron-acetocarmin immediately before crushing. In some cases before fixing a preliminary treatment was made in 0,1 % KOH for a varying length of time. When the cover glass had been removed, the preparations were completely stained in acetocarmin in a 45° C. incubator for one hour for testicles and two hours for embryos.

The ovaries were sectioned after fixing in BOUIN's, FLEMMING's or HERMANN's fluids and stained in HEIDENHAIN's hematoxylin.

As regards *Epiceratodus* and *Protopterus*, sectioned series of embryos, fixed in BOUIN's fluid and stained according to the Azan method, were also examined. These preparations belong to the Zootomic Department of Stockholm University and were originally made for comparative anatomy purposes. Neither the fixing, staining nor the thickness of the sections (8 microns) were therefore suited for cytological investigations. However, they enabled certain comparisons to be made with other species dealt with here.

The optics employed consisted of ZEISS' binocular microscope with LEITZ' objective 112 ×, aperture 1,32 and ZEISS' compens. ocular 15 × and 20 ×. The drawings were made with a camera lucida at a magnification of 5000 × and reduced to about 1500 × for reproduction, when nothing else is stated. The microphotographs were taken with the said objective and a magnification of 1500 ×, except where otherwise stated.

The theory which has been taken as a basis for the interpretation of the various cytological problems raised in the course of this study is that of DARLINGTON (1937). The reasons for this are firstly that a discussion of the results viewed from the angle of different theories would have taken too much space, secondly that, in my opinion, DARLINGTON's view of the mechanism of cell-division is the most

thorough and that which best covers most cytological phenomena, thirdly that it best corresponds with my own results. In certain cases these results involve a confirmation of DARLINGTON's views, in others they can be legitimately interpreted on the basis of those views, in other cases again they serve to supplement them.

The statistical methods used here are those of R. A. FISHER as described in BONNIER and TEDIN (1940).

It should also be mentioned that NOBLE's (1931) systematic classification of Anura and Urodela has been adopted in this work. A short tabular summary of this classification, comprising the species and groups treated or mentioned here, is given below:

Caudata. (In this work termed Urodela.)

Suborder I. Cryptobranchoidea.
Family 1. Hynobiidae.
Hynobius, Pachypalaminus, Salamandrella.

Family 2. Cryptobranchidae.
Cryptobranchus, Megalobatrachus.

Suborder II. Ambystomoidea.
Family 1. Ambystomidae.
Ambystoma.

Suborder III. Salamandroidea.
Family 1. Salamandridae.
Pleurodeles, Triturus.

Family 2. Amphiumidae.
Amphiuma.

Family 3. Plethodontidae.

Suborder IV. Proteida.
Family 1. Proteidae.
Proteus, Necturus.

Suborder V. Meantes.

Family 1. Sirenidae.

Salientia. (In this work termed Anura.)

Suborder I. Amphicoela.

Suborder II. Opistocoela.

Family 1. Discoglossidae.

Alytes, Bombina, Bombinator, Discoglossus.

Family 2. Pipidae.

Xenopus.

Suborder III. Anomocoela.

Family 1. Pelobatidae.

Pelobates, Pelodytes.

Suborder IV. Procoela.

Family 1. Bufonidae.

Bufo.

Family 2. Hylidae.

Hyla.

Suborder V. Diplasiocoela.

Family. Ranidae.

Polypedates, Rana, Rhacophorus.

RESULTS.

I. WITHOUT SPECIAL EXPERIMENTAL TREATMENT.

A. MITOSIS.

In regard to all the species, it may be stated that the mitosis, where it has been investigated at all by previous authors, has been very cursorily dealt with. Though the number of chromosomes in some forty species of Dipnoi, Urodela, Anura, and Chelonia has been ascertained, the exact chromosome morphology is, generally speaking, unknown. The number of chromosomes found by me in the species examined, together with the numbers given by previous authors, is shown in Table 1.

TABLE 1. *Number of chromosomes in the here treated species of Dipnoi, Urodela, Anura, and Chelonia. (A complete list of all the known chromosome numbers in vertebrates is given by* OGUMA *and* MAKINO, *1937.)*

Species	Author's results		Numbers given by previous investigators			Figs.
	2n	n	2n	n	Author	
Dipnoi						
Epiceratodus Forsteri	32—38	—	—	—	—	1; Plate I, Figs.
Protopterus annectens.....	34	17	—	—	—	1, 2; Plate II, Fig. 6
Urodela						
Ambystoma mexicana	28	—	28	—	PARMENTER (1919)	2
			28	14	GALGANO (1933)	
			28	14	CARRICK (1934)	
			28	—	DEARING (1934)	
			24	—	PROKOFIEWA (1935)	
Pleurodeles wallli	24	12	—	12	GALGANO (1933)	18
Triturus cristatus	24	12	—	12	CARNOY et LEBRUN (1899)	3, 17; Plate II, Figs. 1, 2;
			24	12	JANSSENS (1900, 1901, 1902, 1904)	Plate III, Fig. 5
			24	—	JOLLY (1904)	
			24	12	MEEK (1913)	
			24	12	GALGANO (1933)	
» *vulgaris*	24	12	—	12	CARNOY et LEBRUN (1899)	4, 16; Plate II, Figs. 4, 5
			24	—	WALKER (1925)	
			24	12	GALGANO (1933)	
			24	—	PROKOFIEWA (1935)	
			24	12	BÖÖK (1940)	
			24	12	CALLAN (1942)	
Anura						
Bufo calamita	22	11	22	11	STOHLER (1928)	6, 23, 28; Plate II, Fig. 3
» *viridis*	22	11	22	11	BECCARI (1926)	
			22	11	STOHLER (1928)	7, 22, 29
			22	11	GALGANO (1933)	
» *vulgaris*	22	11	22	11	STOHLER (1928)	5, 21, 30
			22	11	IRIKI (1929)	
			22	11	MINOUCHI and IRIKI (1931)	
			—	11	TCHOU SU (1931)	

Species	Author's results		Numbers given by previous investigators			Figs.
	2n	n	2n	n	Author	
Bufo vulgaris (continued)			22	11	GALGANO (1933)	
			24	—	POSKA-TEISS (1933)	
			—	11	WITSCHI (1933)	
Discoglossuspictus	28	—	16	—	CHAMPY (1923)	8
Hyla arborea	24	12	24	12	IRIKI (1930, 1932 b)	9, 20, 31
			—	12	TCHOU SU (1931)	
			24	12	GALGANO (1933)	
Pelobates fuscus...	26	13	—	8	TCHOU SU (1931)	10, 27, 32
Rana arvalis	24	12	22	11	DÜRKEN (1938)	11, 12, 26, 33; Plate III, Fig. 1
» *esculenta* ...	26	13	26	13	DALCQ (1930)	
			26	13	GALGANO (1933)	14, 34
» *temporaria*	26	13	26	13	WITSCHI (1922, 1924)	13, 25, 35
			26	13	MAKINO (1932 a)	
			26	13	GALGANO (1933)	
			26	—	PROKOFIEWA (1935)	
Xenopus laevis ...	36	18	—	—	—	36; Plate II, Fig. 7
Chelonia						
Emys orbicularis	50	25	50	25	MATTHEY (1931)	24, 37; Plate III, Fig. 6

In addition, as regards several species there is some earlier literature, mainly of historical interest. As an exhaustive list of this literature is given by OGUMA and MAKINO (1937), it has not been included here.

As we see, the figures for the number of chromosomes given by previous authors vary from mine as regards several species. PROKOFIEWA (1935) gives 24 or 28 chromosomes in the embryo mitoses of *Ambystoma*, but does not attempt to explain this variation. However, in her description of the chromosomes (*vide infra*), she mentions only twelve pairs, whence she seems to regard that number as typical for the species. As 14 pairs (or 28; cf. p. 262) were found in all the plates I examined and the same figure is given by other investigators (Table 1), I have no hesitation in stating this to be the correct number in *Ambystoma mexicana*. As to whether variations actually occurred in PROKOFIEWA's material — and, if so, how they are to be explained —, I will not hazard an opinion.

The same remark applies to the number given by POSKA-TEISS

(1933) for *Bufo vulgaris*, which has been sharply criticized by SAEZ *et al.* (1936), who consider it to be due to some error.

DÜRKEN (1938) examined *Rana arvalis* together with *R. temporaria* and the hybrid between those species. As regards *R. temporaria* his figures correspond well with those found by the present writer, but for *R. arvalis* he gives the chromosome number $2n = 22$ (Table 1) and, in connection therewith, for the hybrid $2n = 24$. If we examine DÜRKEN's pictures, we see that in *Rana arvalis* DÜRKEN finds only five small pairs in addition to the six large ones, whereas in my material I have invariably found six. Six small bivalents were found also in the several hundred meiosis nuclei I examined (Figs. 26 and 33; Plate III, Fig. 1). The meiosis is not examined by DÜRKEN. I have therefore no hesitation in stating *the number of chromosomes in* the *Rana arvalis* specimens from the Stockholm district which I have examined, *to be* $2n = 24$, $n = 12$.

TCHOU SU (1931) gives $n = 8$ for *Pelobates* and $n = 6$ for *Pelodytes*, but it must be due to some mistake. This investigation was made at early cleavage stages in hybrids. So far as I can see, these low figures are due to the fact that mainly the large chromosomes were discovered.

CHAMPY's (1923) statement ($2n = 16$) regarding the chromosome number in *Discoglossus pictus* must likewise be due to some error. In this species, it must be admitted, I have examined only mitoses and the chromosome number given by me ($2n = 28$) may possibly be inexact. I can, however, definitely state that the number given by CHAMPY does not correspond with that of my material.

In all the species examined, the mitosis completely follows the normal process. In the resting nucleus one or two nucleoli are observed in the diploid species, in the triploids usually three. Nucleolus-forming constrictions have also been discovered in certain species, whilst in others the nucleolus seems to be attached terminally to some chromosome (Scheme 1). Furthermore, a large number of »prochromosomes», *i. e.* heterochromatic parts which in the resting stage are more highly condensed than other chromosome parts (cf. DARLINGTON and LA COUR, 1940; CALLAN, 1942), are observed. They seem to correspond approximately in number to the heterochromatic parts discovered after cold treatment (CALLAN, 1942, and the present paper, p. 307). These »prochromosomes» are more distinct in Dipnoi and Urodela than in Anura, though they are found there, too, in a number approximately corresponding to 2n. During the prophase both relic and relational

coiling (DARLINGTON, 1937) presumably occur (Plate I, Figs. 3 and 4).

The chromosome morphology is shown in Scheme 1.

The values in the scheme represent the average of five good

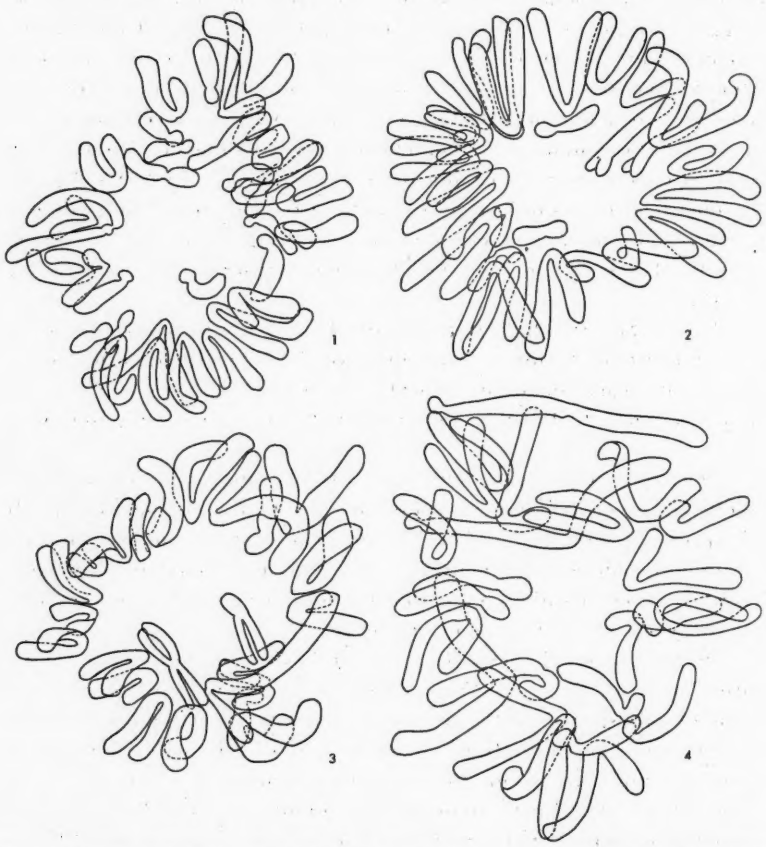
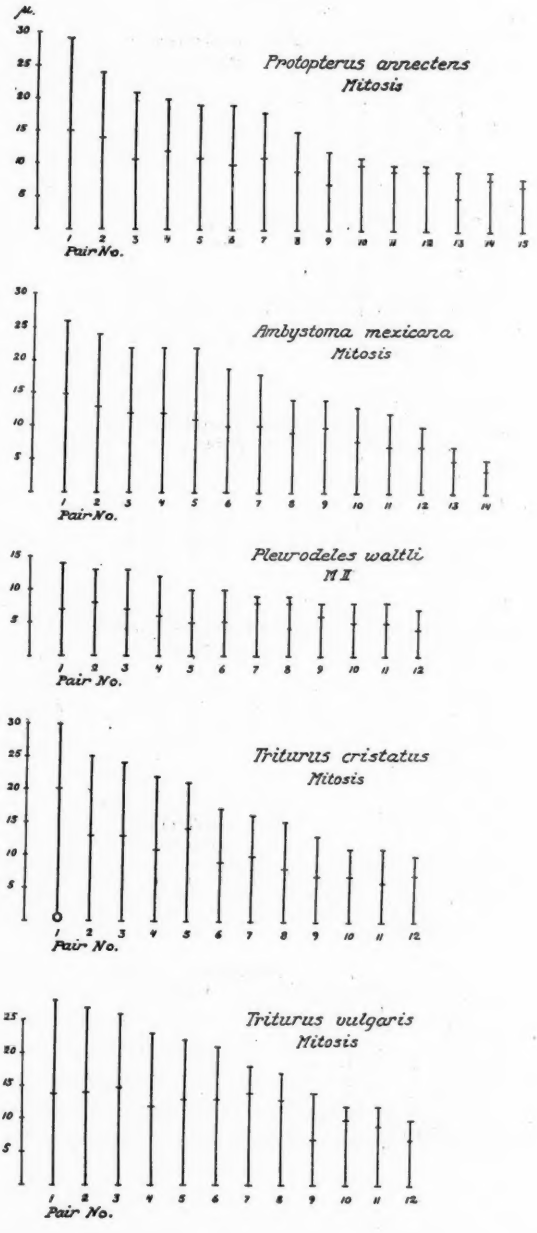


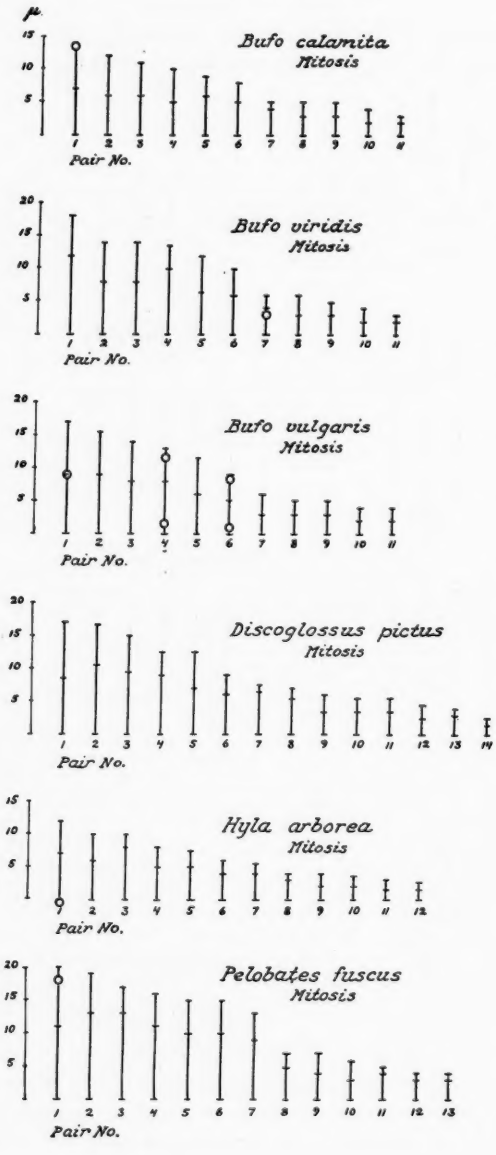
Fig. 1. Mitotic metaphase of *Protopterus annectens.* — Fig. 2. Mitotic metaphase of *Ambystoma mexicana.* — Fig. 3. Mitotic metaphase of *Triturus cristatus.* — Fig. 4. Mitotic metaphase of *Triturus vulgaris.* — (1000 ×.)

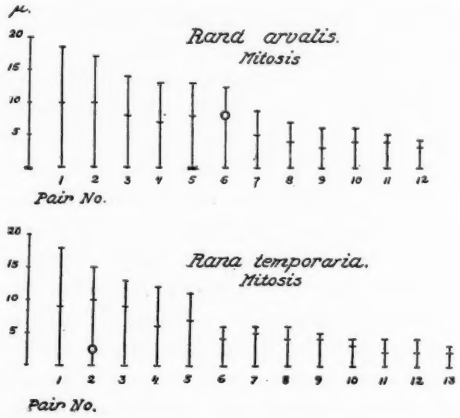
metaphase plates. Generally speaking, no comparison could be made with earlier works, as they do not deal with the more exact chromosome morphology: the only exceptions are PARMENTER (1919) and, to some extent, PROKOFIEWA (1935). The values found by PARMENTER

Scheme 1.


μ.
Protopterus annectens
Mitosis
Pair No.
Ambystoma mexicana
Mitosis
Pair No.
Pleurodeles waltli
M II
Pair No.
Triturus cristatus
Mitosis
Pair No.
Triturus vulgaris
Mitosis
Pair No.

Scheme 1 (continued).





Scheme 1. The chromosome morphology at metaphase in the examined species of Dipnoi, Urodela, and Anura, 1000 ×. (The centromeres are indicated by horizontal lines; the nucleolar constrictions by a circle.)

in *Ambystoma* correspond very well with mine. On the other hand, PROKOFIEWA's statements regarding the chromosome morphology in *Ambystoma*, *Triturus vulgaris* and *Rana temporaria* deviate somewhat from my own findings.

PROKOFIEWA (1935, p. 155) describes the mitotic chromosomes of *Ambystoma* as follows: »I, II and III — three pairs of large chromosomes with arms of equal length . . . IV and V — two pairs of large chromosomes with arms of unequal length . . . VI — a pair of middle-sized chromosomes with arms of equal length . . . VII, VIII, IX and X — four pairs of chromosomes with arms markedly differing in length . . . XI and XII — two pairs of small chromosomes with arms of unequal length . . .».

In this description PROKOFIEWA apparently includes five of pairs 1—6, one of pairs 7—8 and pairs 9—14 (cf. p. 248). Thus interpreted, her results correspond rather well with PARMENTER's findings and my own.

The chromosome pairs of *Triturus vulgaris* are described by PROKOFIEWA (*op. cit.*, p. 152) as follows: »I, II and III — three pairs of large chromosomes with arms of equal length . . . IV and V — two pairs of large chromosomes with unequal arms . . . VI and VII — two pairs of middle-sized chromosomes with arms of equal length . . . VII (VIII), IX and X — three pairs of chromosomes with markedly unequal

arms . . . XI and XII — two pairs of small chromosomes with unequal arms always readily identified in every plate . . .».

CALLAN (1942) groups the chromosomes in this species as follows: »6 M, 2 S, 3 s, 1 m». My own classification, according to the same terminology, would be: 5 M, 3 S, 4 s. As we see, there are certain divergences between the views of PROKOFIEWA, CALLAN and the present writer. In default of facilities for a critical comparison of the material, it is difficult to determine how far these divergences are real. It must be left to coming researches to determine whether cytologically distinguishable local races of this species actually exist.

Rana temporaria has also been investigated by PROKOFIEWA (1935, p. 157). She gives the following description of its chromosome set: »I — a pair of large chromosomes with arms of almost equal length . . . II — a pair of large chromosomes with arms slightly differing in length . . . III and IV — two pairs of chromosomes with arms of equal length . . . V and VI — two pairs of chromosomes with arms markedly differing in length . . . VII — a pair of small chromosomes with arms of almost equal length . . . VIII, IX, X and XI — small (short) chromosomes with arms of markedly unequal length, almost headed . . . XII and XIII — two pairs of extremely small (short) and headed chromosomes».

With this view of *Rana temporaria*'s chromosomes, MAKINO's (1932 a) pictures correspond rather well. There, too, we see six pairs of large chromosomes and seven pairs of small ones, besides which the second pair has arms of almost equal length. In my material there are five large chromosome pairs and the second pair has arms markedly differing in length (Scheme 1).

WITSCHI (1924), like the present writer, finds five large chromosomes and adds (p. 546) that the largest pair has equal arms. This is in good correspondence with my pictures. The same chromosome morphology as in my material is observable also in DÜRKEN's (1938) pictures of *Rana temporaria*'s chromosomes.

These observations seem to point to the existence of at least two cytologically recognizable local races, the one represented by my Swedish, WITSCHI's and DÜRKEN's German forms, the other by PROKOFIEWA's Russian and MAKINO's Japanese forms. The chief differences between them are that the former, the »western form», has five pairs of large chromosomes, the sixth pair with arms of almost equal length and the second pair with arms markedly differing in length with a secondary constriction a few microns from the distal end of the longer arm, whereas the latter, the »eastern form», has six pairs of large chro-

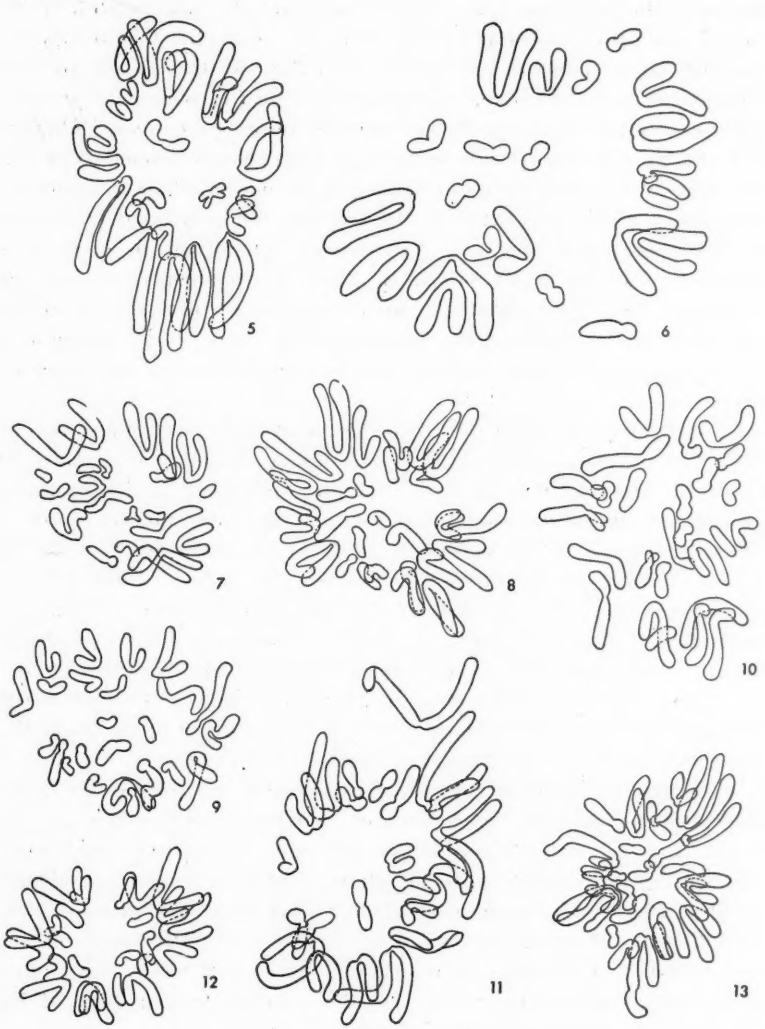


Fig. 5. Mitotic metaphase of *Bufo vulgaris*. — Fig. 6. Mitotic metaphase of *Bufo calamita*. — Fig. 7. Mitotic metaphase of *Bufo viridis*. — Fig. 8. Mitotic metaphase of *Discoglossus pictus*. — Fig. 9. Mitotic metaphase of *Hyla arborea*. — Fig. 10. Mitotic metaphase of *Pelobates fuscus*. — Fig. 11. Mitotic metaphase of *Rana arvalis*. — Fig. 12. Mitotic metaphase of *Rana arvalis*. — Fig. 13. Mitotic metaphase of *Rana temporaria*.

mosomes, the sixth pair with arms of markedly unequal length and the second pair with arms of almost equal length. It is plausible to suppose that the »eastern form» has arisen from the »western form» by unequal segmental interchange between pair 2 and 6, whereby the sixth pair increased in size and the second got more equal arms. Though this, of course, is merely a hypothesis, it is undeniably supported by the fact that the »western form» in its second chromosome has a secondary constriction a few microns from the one end, where a breakage would be liable to occur (Fig. 13; Scheme 1). In regard to such constrictions, CALLAN states (1942, p. 330): »Nevertheless it must be recognized that these segments constitute a source of weakness to the continuity of the chromosome thread and that infrequent breakages at these points would provide the variation necessary for the play of selection».

From Fig. 1 and Scheme 1 it will be seen that the chromosomes at mitotic metaphase in *Protopterus* are two-armed and very long, 5—30 microns. The chromosome lengths form a rather evenly falling curve without any marked steps at any point. The same remarks apply to *Epiceratodus Forsteri*, which — though, owing to unsuitable material, it could not be closely analysed — without the slightest doubt belongs to the same type. The number of chromosomes seems to be about the same as in *Protopterus* (Table 1), the chromosomes are two-armed and the set is not differentiated into groups with marked differences in size.

Lepidosiren paradoxa, examined by AGAR (1911), likewise shows the same characteristics, whence the three now living members of the order Dipnoi show a very good conformity in this respect.

Corresponding types are very common also in Urodela, where the chromosome lengths likewise form an even curve without steps at any point. However, at first sight this group is not so uniform as the Dipnoi. The chromosome numbers in fact show great variations. The highest number, $2n = 64$, is found in *Megalobatrachus japonicus* (IRIKI, 1932 c), lowest, $2n = 18$, in *Proteus* (STIEVE, 1920). Generally speaking, the Cryptobranchoidea have high numbers, 40—64, whereas the Amblystomoidea examined have only 28, the Salamandroidea 24, and the Proteida 18—24 chromosomes (Table 2).

As regards the chromosome morphology there are likewise great variations. In the Cryptobranchoidea rod-shaped chromosomes are usual, but V-shaped ones also occur. In the other suborders the forms examined seem solely to have two-armed chromosomes (Table 2).

It seems to me, however, that the number and morphology of the

TABLE 2. *Chromosome numbers and morphology in Dipnoi, Urodela, and Anura.*

Species	2n	Morphology	Author
Dipnoi			
Epiceratodus Forsteri	32—38	All V	WICKBOM (present paper)
Lepidosiren paradoxa	38	38 V	AGAR (1911, 1912)
Protopterus annectens	34	34 V	WICKBOM (present paper)
Urodela			
Hynobius kimurai (Tango race)	60	16 V, 44 I	SATO (1936 a)
Hynobius lichenatus	58	18 V, 40 I	MAKINO (1932 c)
» 10 sp.	56	20 V, 36 I	MAKINO (1932 c, 1934, 1935 a); SATO (1936 a)
» *retardatus*	40	22 V, 18 I	MAKINO (1932 c)
Pachypalaminus boulengeri	56	20 V, 36 I	SATO (1936 a)
Salamandrella keyserlingii	62	12 V, 50 I	MAKINO (1932 c)
Cryptobranchus alleghenienensis	62	12 V, 50 I	MAKINO (1935 b)
Megalobatrachus japonicus	64	12 V, 52 I	IRIKI (1932 c)
Ambystoma mexicana	28	28 V	*Vide* Table 1
» *punctatum*	28	28 V	CREIGHTON (1938)
Salamandridae 15 sp.	24	24 V	List in OGUMA and MAKINO (1937); WICKBOM (present paper)
Amphiuma means	24	24 V	MCGREGOR (1899)
Proteus anguineus	18	18 V	STIEVE (1920)
Necturus maculosus	24	24 V	KING (1912)
Anura			
Discoglossus pictus	28	28 V	WICKBOM (present paper)
Alytes obstetricans	32	32 V	JANSSENS et WILLEMS (1909)
Bombinator bombinus	24	24 V	GALGANO (1933)
Bombina orientalis	24	24 V	SATO (1939)
Xenopus laevis	36	36 V	WICKBOM (present paper)
Pelobates fuscus	26	26 V	» » »
Bufo 12 sp.	22	22 V	List in OGUMA and MAKINO (1937); WICKBOM (present paper)
Hylidae 7 sp.	24	24 V	IRIKI (1932 b); GALGANO (1933); BUSHNELL *et al.* (1932); WICKBOM (present paper)
Cacopoides tornieri	28	28 V	SATO (1936 b)
Rana 9 sp.	26	26 V	List in OGUMA and MAKINO (1937); WICKBOM (present paper)
» *arvalis*	24	24 V	» » »
Polypedates buergeri	26	26 V	SATO (1934)
Rhacophorus schlegelii	26	26 V	MAKINO (1932 b)

chromosomes in Urodela enable us to trace certain lines of evolution. If we closely examine the conditions in Cryptobranchoidea, we shall find that in the genus *Hynobius* the varying numbers of chromosomes can be brought into correspondence with one another on the basis of ROBERTSON's (1916) »law» that two rod-shaped elements correspond to a V-shaped (SATO, 1936 a). According to SATO, the chromosome numbers in all the *Hynobius* species, except *H. retardatus*, may be derived from a hypothetic number of 2n = 38 two-armed or 76 rod-shaped elements.

SATO's hypothesis, however, can be carried further. *Pachypalaminus boulengeri* corresponds well with the *Hynobius* species (Table 2). The species belonging to the same suborder, *Cryptobranchus allegheniensis* and *Megalobatrachus japonicus* (MAKINO, 1935 b; IRIKI, 1932 c) likewise show good correspondence with this theory, in that the number of rod-shaped elements (1 V = 2 rods), here is 74 or 76 (Table 2). *The chromosome numbers in practically the whole suborder Cryptobranchoidea can thus be derived from 2n=74—76 rod-shaped elements or half as many V-shaped.*

In the chapter on Phylogeny, various reasons are adduced in favour of the view that Urodela are derived from the Dipnoi or from forms closely related to that order.

It is therefore very probable that the suborder Cryptobranchoidea has been differentiated into the present families and species by »fragmentation», that is, by a process in which V-shaped chromosomes, which, phylogenetically speaking, are evidently more primitive, have been split into rod-shaped elements. This process seems to have been carried furthest in *Megalobatrachus japonicus* and *Cryptobranchus allegheniensis*, where merely six pairs of V-shaped elements have been left. The process is thus paralleled by that which ROBERTSON (1916) observed in grasshoppers. In reptiles MATTHEY (1931) attempted to show that the number of chromosomes in all the forms examined may be derived from n = 24 rods, by the theory that the reverse process, »fusion», had taken place during the phylogeny.

Corresponding conditions have been found by STURTEVANT and NOVITSKY (1941) in *Drosophila*. Here the chromosome sets in the different species seem to have been formed by combinations of rod-shaped elements into varying numbers of V-shaped and rod-shaped forms. These authors consider that the *Drosophila virilis* type, where all the elements form free rods, is the most primitive.

The view that such chromosome changes have played an im-

portant part in the phylogeny of Urodela is borne out by the existence of extensive heterochromatic parts round the centromeres (cf. STURTEVANT and NOVITSKY, 1941).

On the phylogenetic connection between the suborders of Urodela the cytological conditions do not throw much light. NOBLE (1931, p. 471) states on comparative anatomic grounds: »The salamanders of the family *Ambystomidae* have apparently arisen from Hynobiids or from prohynobiids», and (p. 473) regarding Salamandroidea: »... seems to have arisen independently from prohynobiid ancestors not living today». According to NOBLE (*op. cit.*), Proteida are derived from Salamandroidea. These views are shared also by HERRE (1935), though he introduces Proteida as the most specialized group within the Salamandroidea.

If these views are correct, the said suborders must have been differentiated before the said fragmentation in Hynobiidae or their ancestors commenced. It seems, instead, as if the number of chromosomes in Ambystomoidea, Salamandroidea, and Proteida had been reduced as compared with that of the Dipnoi. This can be explained by a series of »fusions». [The said »fusions» and »fragmentations», of course, should not be interpreted too literally, as in that case certain difficulties will arise in regard to the centromeres. A way of escape from these difficulties by the assumption of unequal segmental interchanges has been indicated by DARLINGTON (1937, p. 559).] The higher chromosome number in *Ambystoma* may possibly be interpreted in favour of NOBLE's view that this genus is more closely related to Cryptobranchoidea than are the Salamandroidea, that is to say, is more primitive. *Proteus* is further modified in the direction of a reduced chromosome number (STIEVE, 1920).

A somewhat different cytological type is found in Anura. The length of the chromosomes is still considerable, though less than in the forms previously dealt with (Scheme 1). It is also noteworthy that the chromosome lengths of practically all the Anura examined form a curve with a steep slope in the middle (Scheme 1).

Similar observations in regard to these forms have been made by earlier authors. Nine American and East Asiatic *Bufo* species, examined by IRIKI (1929), MINOUCHI and IRIKI (1931), MAKINO (1932 a), WITSCHI (1933), SAEZ *et al.* (1936), and SATO (1936 b), as well as the three European forms examined here, which have been dealt with also by BECCARI (1926), STOHLER (1928), and GALGANO (1933), all have 6 large

chromosome pairs, separated from the 5 small ones by a step in the length curve.

Bombina orientalis and *Bombinator pachypus* have 7 large pairs and 5 small ones, whence the said step in the curve has been observed also in Discoglossidae (GALGANO, 1933; SATO, 1939). However, its place is after the seventh pair, and not, as in *Discoglossus*, after the fifth. The possible connection of this fact with the different chromosome numbers in these forms is discussed below.

The same numbers of small and large chromosomes as those observed by the author in *Hyla arborea* are reported by IRIKI (1932 b) from *Hyla arborea japonica*, by GALGANO (1933) in the European variety, and by BUSHNELL *et al.* (1939) in 5 American Hylids.

Ranidae have been examined by several investigators. In *Rana esculenta* GALGANO (1933) also finds 5 large chromosomes well distinguished from the 8 small ones. The same results as those arrived at by the author in regard to the number of large and small chromosomes in *Rana temporaria* have been found also by WITSCHI (1924) and as regards this species and *R. arvalis* by DÜRKEN (1938). — DÜRKEN, however, gives only 11 pairs in *R. arvalis* (cf. p. 249). — In regard to *R. temporaria*, PROKOFIEWA (1935) and MAKINO (1932 a), however, find 6 large pairs and 7 small ones. The cause of these divergences have already been discussed. Among other Ranids, *Rana limnocharis* (SATO, 1934), *nigromaculata* (IRIKI, 1932 a), *pipiens* (SWINGLE, 1917; PARMENTER, 1920, 1925), *rugosa* (IRIKI, 1932 a), *Polypedates buergeri* (SATO, 1934), and *Rhacophorus schlegelii* (MAKINO, 1932 b) have 5 large pairs besides the 8 small ones. All the Ranids except *Rana arvalis* and certain *temporaria* forms thus seem to have the step in the chromosome curve after the fifth pair.

If we consider these conditions within the different suborders, we shall find that there is a certain connection between the variations in the place of this step in the chromosome curve and the number of chromosomes. In *Discoglossidae* the *Bombina* (*Bombinator*) species have $n = 12$ and the step after the seventh pair, whilst *Discoglossus* has $n = 14$ and the step after the fifth pair. It is very plausible to assume a real causal connection. For the present, however, we must content ourselves with explaining this observation by »fusions» or »fragmentations» (DARLINGTON, 1937, p. 559), whereby large chromosomes have been converted into a larger number of small ones or *vice versa*.

Which of these types is the primary one, *Discoglossus* or *Bombina*,

it is difficult to determine, but there are certain indications that *Discoglossus* is the more primitive type (*vide infra*).

As regards Procoela, the *Bufo* species have the step after the sixth pair and 11 pairs of chromosomes, whilst the Hylids have 12 pairs and the step after the fifth. This corresponds well with the above statements regarding Discoglossidae.

There are similar examples also in Diplasiocoela. *Rana arvalis* has $n = 12$ and the step after the sixth pair, *R. temporaria* (the »western form») and all the other *Rana* species examined have $n = 13$ and the step after the fifth pair. On the other hand, that the variation in the

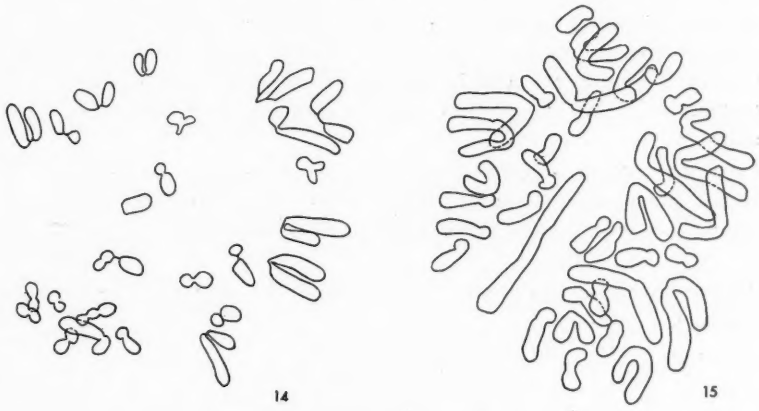


Fig. 14. Mitotic metaphase of *Rana esculenta*. Cold treatment. — Fig. 15. Mitotic metaphase of *Rana esculenta* $3n - 1$.

place of the step need not necessarily be connected with a change in the number of chromosomes is shown by a comparison between the »eastern» and »western» form of *Rana temporaria*, which both have $n = 13$ but the step at different places in the set (cf. p. 254).

As regards the number of chromosomes, Anura are far more uniform than Urodela. The number fluctuates between $2n = 36$ in *Xenopus* and $2n = 22$ in the *Bufo* species. *Also in Anura it is a striking fact that the highest numbers have been found in the most primitive groups* (Table 2).

The occurrence of high numbers in the most primitive group and lower in the others may possibly imply that in Anura »fusions» have played an important part in the phylogeny.

Abnormal chromosome numbers.

In triploid *Rana esculenta* a number of spermatogonium divisions have been analysed. In the cases observed, the divisions apparently proceed quite normally but the chromosome numbers indicate that this is not invariably the case. The majority of the cells have the complete triploid chromosome number 3n = 39, but several nuclei with 3n — 1 = = 38 (Fig. 15) have been observed. In one case such a low chromosome number as 3n — 6 = 33 has actually been found.

Moreover, stray polyploid cells have been found in otherwise diploid organisms. In *Ambystoma mexicana*, *Triturus cristatus*, *T. vulgaris*, *Bufo vulgaris*, *Discoglossus pictus*, *Pelobates fuscus*, *Rana arvalis*, and *R. temporaria* tetraploid cells have been met with in embryos. In *Triturus vulgaris*, *Bufo vulgaris*, *Rana arvalis*, and *R. temporaria* tetraploid cells have been found in testicles (spermatogonium divisions). In *Protopterus* a group of octoploid spermatogonium cells have been observed.

These polyploid cells appear to have arisen by syndiploidy. A similar phenomenon has previously been found in many organisms. Among the lower vertebrates, syndiploidy has been observed in *Teleostei* by WICKBOM (1943).

In regard to the origin of triploids in Urodela and Anura, *vide* p. 288.

B. MEIOSIS.

a. Diploids.

As previously indicated, the meiosis has been examined only in testicles; all my observations therefore refer to meiosis in the *male*. In all the forms studied, the meiosis on the whole follows the normal course. A few peculiarities, however, call for mention here. A polarization of the chromosome ends can already be discerned at leptotene, and becomes very marked at zygotene and pachytene. All the chromosome ends gather towards a point at the periphery of the nucleus, whilst the remaining parts of the chromosomes coil through the nucleus in the form of long loops. Similar observations have been made as regards these forms by earlier authors [JANSSENS (1900) and WALKER (1925) in *Triturus*; AGAR (1911) in *Lepidosiren*; WITSCHI (1924) in *Rana*; STOHLER (1928) and SAEZ *et al.* (1936) in *Bufo* species]. Owing to the difference in size between the two chromosome groups (see p. 259) in Anura, it is rarely possible at zygotene and pachytene,

even in very good preparations, to observe the whole haploid chromosome number: as a rule only the large chromosome pairs can be discerned. The small chromosome pairs are so short that they with both ends fixed at the same point do not extend so far out into the nucleus that they can be distinguished in the tangle at the pole.

In good preparations it can be observed that at *zygotene the chromosomes are paired at the distal ends, whilst in other parts they are still unpaired.* Such observations have previously been made as regards these forms (WITSCHI, 1924; CALLAN, 1942), as well as in *Lepidosiren* (AGAR, 1911). This shows that the *zygotene pairing in all the species dealt with here begins at the polarized ends*, which indeed seems to be the general rule in those organisms where polarization occurs (DARLINGTON and DARK, 1932; DARLINGTON, 1937, p. 91). *As this matter is of fundamental importance for a thorough understanding of the meiosis in Dipnoi, Urodela, Anura, and Emys, especially the peculiar details in the frequency and distribution of the chiasmata,* it must be specially stressed here.

The earliest diplotene stages, especially in Anura, are rather diffuse and therefore elude observation. Somewhat later stages, on the other hand, give very distinct pictures, which facilitate exact analyses of the chiasma frequency and distribution (Figs. 16—27). At diplotene and later stages, interlocking between bivalents has been observed in Anura and Urodela (Figs. 18—21, 23, 25, and 26). This, however, is far more common in Anura than in Urodela. Although the actual number has not been counted, it may be estimated that about half of the nuclei in Anura have two or more bivalents interlocked. Owing to the localization of the chiasmata, the interlocking in all the cases observed by the present writer in Anura was proximal; in Urodela a few cases of distal interlocking have been observed.

The diakinesis differs very considerably in different groups. In Urodela and Dipnoi the diplotene spiralization successively increases, the chiasmata are terminalized, and the chromosomes pass into this phase gradually. In Anura and Emys, on the other hand, a new spiralization sets in at the very beginning of the diakinesis, namely the major spiral (DARLINGTON, 1937), whence the diakinetic and M^I chromosomes differ considerably in appearance from the diplotene and mitotic chromosomes. The family Discoglossidae seems to form an exception in this respect: to judge from the pictures published by JANSSENS et WILLEMS (1909), CHAMPY (1923), GALGANO (1933), and SATO (1939), all the four species examined have approximately the

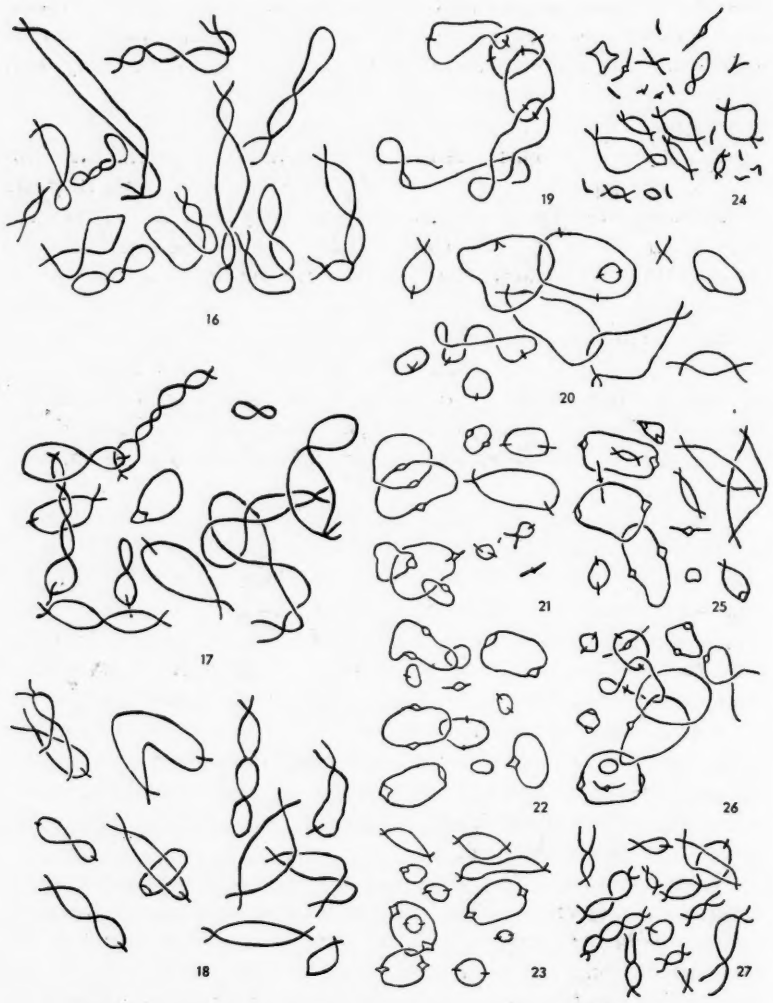


Fig. 16. Diplotene of *Triturus vulgaris.* — Fig. 17. Diplotene of *Triturus cristatus.* — Fig. 18. Diplotene of *Pleurodeles waltli* $2n + 2$. — Fig. 19. Diplotene of *Hyla arborea.* Chain of 4 interlocked bivalents. — Fig. 20. Diplotene of *Hyla arborea.* — Fig. 21. Diplotene of *Bufo vulgaris.* N. B. interlocking. — Fig. 22. Diplotene of *Bufo viridis.* — Fig. 23. Diplotene of *Bufo calamita.* N. B. interlocking. — Fig. 24. Diplotene of *Emys orbicularis.* — Fig. 25. Diplotene of *Rana temporaria.* N. B. interlocking. — Fig. 26. Diplotene of *Rana arvalis.* N. B. interlocking. — Fig. 27. Diplotene of *Pelobates fuscus.*

same chromosome lengths at mitosis and meiosis. In *Alytes* (JANSSENS et WILLEMS, *op. cit.*) this does not always seem to be the case. A mitotic appearance of the chromosomes at M^I, *i. e.* minor spirals only, has been

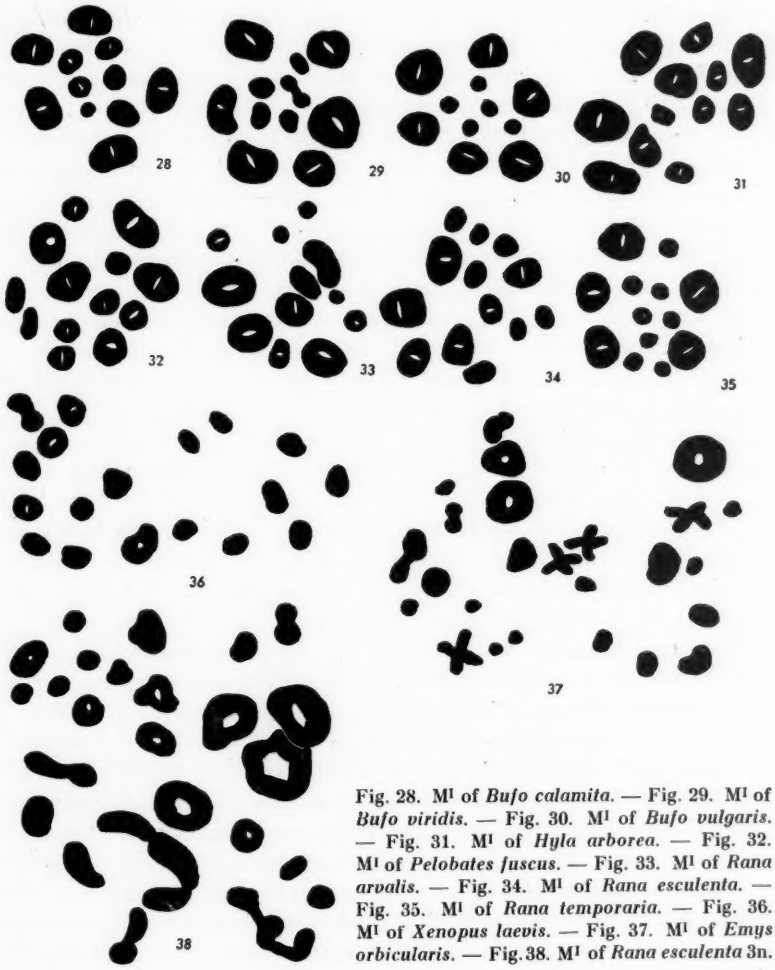


Fig. 28. M^1 of *Bufo calamita*. — Fig. 29. M^1 of *Bufo viridis*. — Fig. 30. M^1 of *Bufo vulgaris*. — Fig. 31. M^1 of *Hyla arborea*. — Fig. 32. M^1 of *Pelobates fuscus*. — Fig. 33. M^1 of *Rana arvalis*. — Fig. 34. M^1 of *Rana esculenta*. — Fig. 35. M^1 of *Rana temporaria*. — Fig. 36. M^1 of *Xenopus laevis*. — Fig. 37. M^1 of *Emys orbicularis*. — Fig. 38. M^1 of *Rana esculenta* 3n.

observed by GALGANO (1933) in some cells of *Rana esculenta* in otherwise normal testicles. A similar observation has been made by SWINGLE (1925) as regards *Rana catesbiana* at the first larval meiosis. So far as

I can find, *Urodela, Dipnoi, and the Discoglossids are the only known cases in any organism where major spirals are normally missing* (cf. KUWADA, 1939, p. 232). (KUWADA adds about meiotic chromosomes without major spiral: »May possibly be found in animals».) This fact seems particularly remarkable if we consider the immense chromosome length in these forms, which certainly have the longest M^I bivalents ever examined. That the spiralization conditions are as above stated is indicated by the following circumstances. Firstly, the major spiral in Anura and *Emys*, when uncoiled by treatment with KOH, was found to consist of chromatids of the usual mitotic size (Fig. 46). Since the mitotic chromatids by uncoiling with KOH were found to consist of a close spiral (Plate III, Fig. 7; Figs. 40 and 41), it may be presumed that this is also the case with the meiotic chromosomes freed from the major spiral. Secondly, the chromosome length in Dipnoi and Urodela is about the same in meiosis and mitosis, which could scarcely be explained if there were an additional spiral in the meiotic chromosome, whereas in Anura and *Emys* the mitotic length is two to four times as large as the meiotic length at metaphase. Thirdly, in Anura the secondary constrictions completely disappear after diplotene, whereas they are retained in Urodela. (Such constrictions have not been discovered in *Emys*.)

The metaphase follows the usual course, with the modifications entailed by the special spiralization conditions in Urodela and Dipnoi and the localization of the chiasmata (Plate II, Figs. 2, 3, 4, 6, and 7; Figs. 28—38). The anaphase and the second division proceed normally. In the second division, Anura have both a major and a minor spiral; Urodela and Dipnoi only the minor. Later stages in the spermatogenesis have not been investigated.

Chiasma frequency and localization. (Diploid species.)

The chiasma frequency in different species at different stages, as well as other data connected therewith, have been summarized in Tables 3 and 4.

Certain details in Tables 3—4 call for special comment. An examination of the number of X:ta/bivalent at diplotene in the various groups shows that statistically significant differences exist between the different Urodela ($P < 0{,}001$), whereas Anura are fairly homogeneous ($0{,}05 > P > 0{,}02$). Between Anura and Urodela there is a marked difference ($P < 0{,}001$). *Emys*, at any rate in the large chromosomes, corresponds closely to Anura.

TABLE 3. *Chiasma frequency at diplotene in Protopterus, Urodela, Anura, and Emys.*

Species	Number of bivalents with						Total bivalents	Number of nuclei
	1 X	2 X:ta	3 X:ta	4 X:ta	5 X:ta	6 X:ta		
Dipnoi								
Protopterus annectens[1]	3	29	18	14	4	—	68	4
Urodela								
Pleurodeles waltli	—	79	21	8	—	—	108	9
Triturus cristatus	1	72	84	60	21	2	240	20
» *vulgaris*	2	32	20	3	2	1	60	5
Anura								
Bufo calamita	11	207	2	—	—	—	220	20
» *viridis*	18	201	1	—	—	—	220	20
» *vulgaris*	6	211	3	—	—	—	220	20
Hyla arborea	22	214	4	—	—	—	240	20
Pelobates fuscus	38	212	9	1	—	—	260	20
Rana arvalis	13	224	3	—	—	—	240	20
» *esculenta*	20	237	3	—	—	—	260	20
» *temporaria*	9	121	—	—	—	—	130	10
Xenopus laevis	27	326	7	—	—	—	360	20
Emys orbicularis[2]	13	68	7	—	—	—	88	8

[1] Metaphase. — [2] The 11 largest bivalents.

As the chiasma frequency is to some extent correlated to the chromosome length (*v. infra*), which is considerably larger in *Protopterus* and Urodela, a special analysis has been made for the purpose of ascertaining whether the chiasma frequency in chromosomes of equal length in Anura and Urodela shows any divergence. The material consisted of the six small chromosome pairs in five diplotene cells of *Triturus vulgaris*, on the one hand, and the seven large pairs in twenty diplotene cells of *Pelobates fuscus*, on the other. The values are summarized on p. 269. Chromosomes of approximately equal length (10—20 microns) from the species of Anura which has the highest chiasma frequency have been compared with those of the Urodelian which shows the lowest frequency.

An analysis of variance of the difference in the number of chiasmata/bivalent between *Pelobates* and *Triturus* gives $P < 0{,}001$.

The difference in chiasma frequency between Urodela and Anura

TABLE 4. *Chiasma frequencies and terminalization in Protopterus, Urodela, Anura, and Emys.*

Species	Stage	Number of nuclei	Number of X:ta	X:ta per bivalent	Number of terminal X:ta	Terminalization coefficient
Protopterus annectens	Metaphase	4	191	2,81	111	0,58
Pleurodeles waltli	Diplotene	9	253	2,34	35	0,14
» »	Diakinesis	8	211	2,20	94	0,45
» »	Metaphase	20	527	2,20	339	0,64
Triturus cristatus	Diplotene	20	754	3,14	146	0,19
» »	Diakinesis	20	722	3,01	289	0,40
» »	Metaphase	20	704	2,94	331	0,47
» *vulgaris*	Diplotene	5	154	2,57	35	0,23
» »	Diakinesis	9	216	2,00	82	0,38
» »	Metaphase	9	212	1,97	114	0,54
Bufo calamita	Diplotene	20	431	1,96	212	0,49
» *viridis*	Diplotene	20	423	1,92	214	0,51
» »	Metaphase	20	423	1,92	414	0,98
» *vulgaris*	Diplotene	20	437	1,99	269	0,62
» »	Diakinesis	20	427	1,94	366	0,85
» »	Metaphase	20	425	1,93	422	0,99
Hyla arborea	Diplotene	20	462	1,93	123	0,27
» »	Diakinesis	20	466	1,94	342	0,73
» »	Metaphase	20	463	1,93	451	0,97
Pelobates fuscus	Diplotene	20	493	1,89	93	0,19
» »	Diakinesis	20	482	1,85	216	0,45
» »	Metaphase	20	480	1,85	426	0,89
Rana arvalis	Diplotene	20	470	1,96	297	0,63
» »	Diakinesis	20	451	1,88	379	0,84
» »	Metaphase	20	444	1,85	437	0,98
» *esculenta*	Diplotene	20	503	1,93	310	0,62
» *temporaria*	Diplotene	10	251	1,93	159	0,63
» »	Diakinesis	20	503	1,93	466	0,93
» »	Metaphase	20	496	1,91	493	0,99
Xenopus laevis	Diakinesis	20	700	1,94	573	0,82
» »	Metaphase	20	701	1,95	641	0,91
Emys orbicularis	Diakinesis	8 [1]	170	1,93	129	0,76

[1] Only the 11 large bivalents.

also as regards chromosomes of approximately equal length is thus statistically established.

A marked terminalization of the chiasmata has been found in all the species (Table 4). With the exception of *Pelobates*, it is throughout most noticeable between diplotene and diakinesis, whereas between

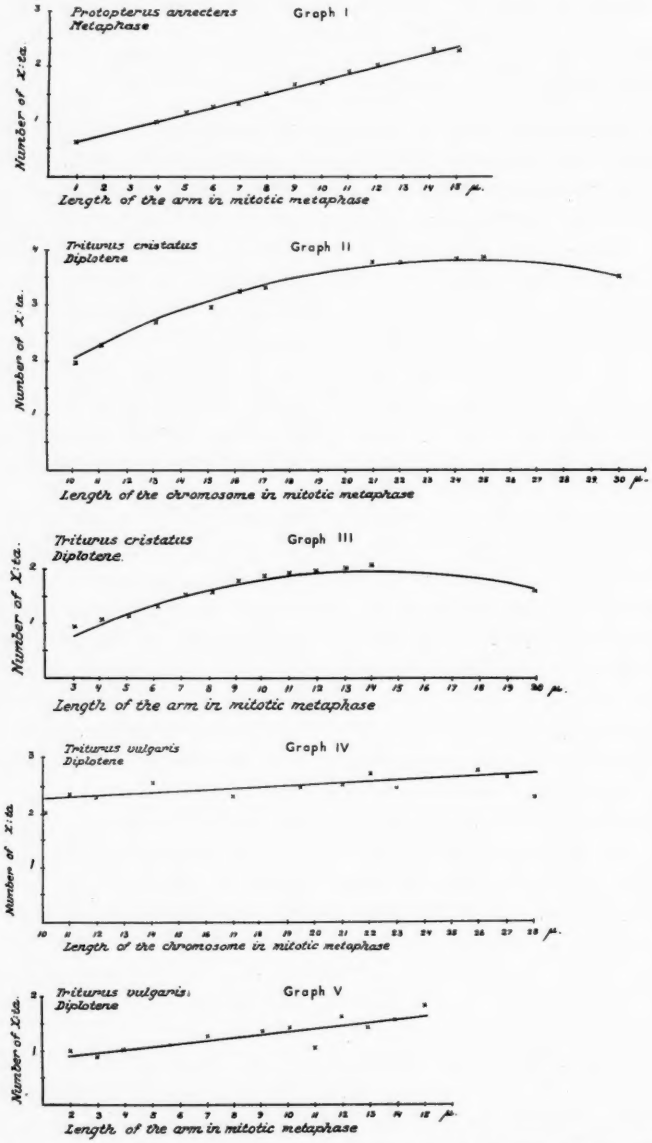
Species	Number of bivalents with						
	1 X	2 X:ta	3 X:ta	4 X:ta	5 X:ta	Total biv.	Mean X:ta/biv.
Pelobates fuscus.....	1	129	9	1	—	140	2,07
Triturus vulgaris ...	1	19	7	2	1	30	2,43

diakinesis and metaphase it is, generally speaking, very slight. There is a distinct difference in the degree of terminalization between Anura and *Emys*, on the one hand, and *Protopterus* and Urodela on the other. The difference is observable at all stages, but is most marked at metaphase, where the first mentioned forms have a terminalization coefficient varying, according to the species, between 0,89 and 0,99, while the last-mentioned forms have a coefficient between 0,47 and 0,65; this signifies that at metaphase almost all the chiasmata are terminalized in Anura, but merely about half of them in Urodela and *Protopterus*. In this respect Anura correspond to *Primula* and *Campanula* (see DARLINGTON, 1937, pp. 105 and 514), whilst Urodela hold an intermediate position between this extreme and the opposite one, which is represented by *Fritillaria* (DARLINGTON, *loc. cit.*).

In regard to the conditions which give rise to these differences, DARLINGTON states (1937, p. 512): »The differences found in degree of terminalization in organisms with chromosomes of similar sizes . . . may be said to be immediately due to differences in the rate of movement relative to external development». That is to say, a higher degree of terminalization may, in some cases, be due to a slow rate of development in the cells, so that the chiasmata have more time to become terminalized. This agrees well with STRAUB's (1936) observations, which the present writer can confirm (p. 306), that the degree of terminalization falls at high temperature and rises at low.

A very good example of this fast development of the cell is *Pelobates* which also deviates from the other forms, in that spiralization does not reach its maximum till metaphase, instead of, as usual, at diakinesis. Subsequently, however, SWANSON (1941) showed that the chiasma frequency and the number of major coils are negatively correlated, and that the degree of terminalization increases with the number of major coils. As SWANSON's investigations are accessible merely in a summary, further particulars on this matter are not obtainable at present. It is, however, plausible to suppose that the degree of spiralization is not only a resultant of the same forces which influence the terminalization, but also itself affects it, possibly by increased »body repulsion» [a term introduced by DARLINGTON (1937)].

It must, however, be recognized that the factors indicated do not



Graphs I—V. The relation between chromosome (arm) length and number of chiasmata. (Cf. Table 5.)

suffice to explain all the differences in the degree of terminalization between Anura and Urodela, nor, of course, between other organisms with marked divergences in this regard. It should, however, be added that differences of another kind evidently play an important part, namely those which are due to different physiological or morphological properties, such as varying degrees of repulsion between chromosomes or centromeres, etc. In regard to the rôle played by these forces in terminalization, see DARLINGTON (1937, p. 514 ff.).

The connection between chromosome (arm) length and chiasma frequency.

Where possible, the relation between the chromosome (and arm) length and the number of chiasmata in the various species has been computed. The results are summarized in Table 5 and Graphs I—V.

The conditions in *Pleurodeles* could not be analysed owing to the lack of good mitotic pictures for the exact determination of the chromosome lengths. From Table 5 it appears, however, that *Pleurodeles* must correspond most closely with *Triturus cristatus*, as the curve for the relation of the number of chiasmata to the chromosome length seems to follow a quadratic equation (*v. infra*).

A close examination of *Xenopus* and *Emys* was likewise impeded by the lack of good mitotic pictures; moreover, the chromosomes of *Xenopus* are so small and so like one another that there is no possibility

TABLE 5. *The relation between the number of chiasmata and the length of chromosomes and chromosome arms at mitotic metaphase.*

a. *Protopterus annectens.* Metaphase.

Number of X:ta	Length of chromosome in microns													Total
	5	8	9	10	11	12	15	18	19	20	21	24	29	
5											1	1	2	4
4						1	1	1	1	3	2	3	2	14
3	1	3	1	1	1		1	2	6	1	1			18
2	3	3	7	6	3	3	2	1	1					29
1		2		1										3
Frequency	4	8	8	8	4	4	4	4	8	4	4	4	4	68
S(y)	9	17	17	16	9	10	11	12	24	15	16	17	18	191
$\bar{y}$	2,25	2,13	2,13	2,00	2,25	2,50	2,75	3,00	3,00	3,75	4,00	4,25	4,50	2,8088

Equation of the regression line: The scanty material does not admit of accurate analysis.

Number of X:ta	Length of arm in microns												Total
	1	4	5	6	7	8	9	10	11	12	14	15	
3									1		2	1	4
2		1	1	1	6	6	10	11	9	4	6	3	58
1	18	6	7	3	10	6	6	5	2				63
0	10	1											11
Frequency	28	8	8	4	16	12	16	16	12	4	8	4	136
S(y)	18	8	9	5	22	18	26	27	23	8	18	9	191
$\bar{y}$	0,64	1,00	1,13	1,25	1,37	1,50	1,63	1,69	1,92	2,00	2,25	2,25	1,4044

$Y = 0{,}1215x + 0{,}5217$. Cf. Graph I.

b. *Pleurodeles waltli.* Diplotene.

Number of X:ta	Chromosome pair No.												Total
	12	11	10	9	8	7	6	5	4	3	2	1	
4						1			2	3	1	1	8
3						3	2	3	4	3	4	2	21
2	9	9	9	9	9	5	7	6	3	3	4	6	79
Frequency	9	9	9	9	9	9	9	9	9	9	9	9	108
S(y)	18	18	18	18	18	23	20	21	26	27	24	22	253
$\bar{y}$	2,00	2,00	2,00	2,00	2,00	2,56	2,22	2,33	2,89	3,00	2,67	2,44	2,3426

As it was not possible to observe the mitotic lengths, the relation cannot be determined, but the regression line seems to be determined by a quadratic equation.

c. *Triturus cristatus.* Diplotene.

Number of X:ta	Length of chromosome in microns											Total
	10	12	13	15	16	17	21	22	24	25	30	
6						1	1					2
5						2	5	2	7	3	2	21
4		2	1	5	6	4	6	12	5	12	7	60
3	1	11	11	9	12	8	5	6	6	5	10	84
2	18	27	8	6	2	5	3		2		1	72
1	1											1
Frequency	20	40	20	20	20	20	20	20	20	20	20	240
S(y)	40	95	53	59	64	66	76	76	77	78	70	754
$\bar{y}$	2,00	2,38	2,65	2,95	3,20	3,30	3,80	3,80	3,85	3,90	3,50	3,1417

$Y = 0{,}4207x - 0{,}0086x^2 - 1{,}3015$. Cf. Graph II.

Number of X:ta	Length of arm in microns													Total
	3	4	5	6	7	8	9	10	11	12	13	14	20	
3					1	2	2	1	6	1	6	6	1	26
2		1	2	17	39	21	9	31	43	17	26	9	8	223
1	19	19	18	43	60	17	9	8	11	2	8	5	11	230
0	1													1
Frequency	20	20	20	60	100	40	20	40	60	20	40	20	20	480
S(y)	19	21	22	77	141	65	33	73	115	39	78	41	30	754
$\bar{y}$	0,95	1,05	1,10	1,28	1,41	1,63	1,65	1,83	1,91	1,95	1,95	2,05	1,50	1,5708

$Y = 0{,}2773x - 0{,}0100x^2 + 0{,}0255$. Cf. Graph III.

d. *Triturus vulgaris*. Diplotene.

Number of X:ta	Length of chromosome in microns											Total
	10	12	14	17	18	21	22	23	26	27	28	
6							1					1
5					1					1		2
4		2				1						3
3			3	3	2	1	1	3	4	1	2	20
2	5	7	2	2	2	3	2	2	1	3	3	32
1		1					1					2
Frequency	5	10	5	5	5	5	5	5	5	5	5	60
S(y)	10	23	13	13	15	13	14	13	14	14	12	154
$\bar{y}$	2,00	2,30	2,60	2,60	3,00	2,60	2,80	2,60	2,80	2,80	2,40	2,5667

$Y = 0{,}0303x - 0{,}0001x^2 + 2{,}0119$. Cf. Graph IV.

Number of X:ta	Length of arm in microns											Total
	2	3	4	7	9	10	11	12	13	14	15	
4										1		1
3					2	1			1	1		5
2				3	2			3	6	5	4	23
1	5	9	10	12	10	4	10	2	13	13	1	89
0		1			1							2
Frequency	5	10	10	15	15	5	10	5	20	20	5	120
S(y)	5	9	10	18	20	7	10	8	28	30	9	154
$\bar{y}$	1,00	0,90	1,00	1,20	1,33	1,40	1,00	1,60	1,40	1,50	1,80	1,2833

$Y = 0{,}0489x + 0{,}8126$. Cf. Graph V.

e. *Bufo calamita.* Diplotene.

Number of X:ta	Length of chromosome in microns									Total
	3	4	5	8	9	10	11	12	13	
3				1				1		2
2	13	19	60	18	19	19	20	19	20	207
1	7	1		1	1	1				11
Frequency	20	20	60	20	20	20	20	20	20	220
S(y)	33	39	120	40	39	39	40	41	40	431
$\bar{y}$	1,65	1,95	2,00	2,00	1,95	1,95	2,00	2,05	2,00	1,9591

The values are not satisfied either by a linear or by a quadratic equation.

Number of X:ta	Length of arm in microns							Total
	1	2	3	4	5	6	7	
2					1	1		2
1	36	96	78	20	78	99	20	427
0	4	4	2		1			11
Frequency	40	100	80	20	80	100	20	440
S(y)	36	96	78	20	80	101	20	431
$\bar{y}$	0,90	0,96	0,98	1,00	1,00	1,01	1,00	0,9795

The values are not satisfied either by a linear or by a quadratic equation.

f. *Bufo viridis.* Diplotene.

Number of X:ta	Length of chromosome in microns									Total
	3	4	5	6	10	12	13,5	14	18	
3									1	1
2	9	18	19	39	19	20	20	39	18	201
1	11	2	1	1	1			1	1	18
Frequency	20	20	20	40	20	20	20	40	20	220
S(y)	29	38	39	79	39	40	40	79	40	423
$\bar{y}$	1,45	1,90	1,95	1,98	1,95	2,00	2,00	1,98	2,00	1,9227

The values are not satisfied either by a linear or by a quadratic equation.

Number of X:ta	Length of arm in microns									Total
	1	2	3	4	5	6	8	10	12	
2									1	1
1	10	95	60	59	20	99	40	20	18	421
0	10	5		1		1			1	18
Frequency	20	100	60	60	20	100	40	20	20	440
S(y)	10	95	60	59	20	99	40	20	20	423
$\bar{y}$	0,50	0,95	1,00	0,98	1,00	0,99	1,00	1,00	1,00	0,9614

The values are not satisfied either by a linear or by a quadratic equation.

g. *Bufo vulgaris.* Diplotene.

Number of X:ta	Length of chromosome in microns								Total
	4	5	6	8	12	14	16	17	
3					1		1	1	3
2	39	37	19	20	38	20	19	19	211
1	1	3	1		1				6
Frequency	40	40	20	20	40	20	20	20	220
S(y)	79	77	39	40	80	40	41	41	437
$\bar{y}$	1,98	1,93	1,95	2,00	2,00	2,00	2,05	2,05	1,9864

$Y = 0{,}0073x + 1{,}9180.$

Number of X:ta	Length of arm in microns							Total
	2	3	4	5	6	8	9	
2						1	2	3
1	135	60	20	59	60	59	38	431
0	5			1				6
Frequency	140	60	20	60	60	60	40	440
S(y)	135	60	20	59	60	61	42	437
$\bar{y}$	0,96	1,00	1,00	0,98	1,00	1,02	1,05	0,9932

$Y = 0{,}0092x + 0{,}9505.$

h. *Hyla arborea.* Diplotene.

Number of X:ta	Length of chromosome in microns									Total
	2,5	3	4	5	6	7	8	10	12	
3							1	1	2	4
2	7	16	60	20	19	20	19	39	14	214
1	13	4			1				4	22
Frequency	20	20	60	20	20	20	20	40	20	240
S(y)	27	36	120	40	39	40	41	81	38	462
$\bar{y}$	1,35	1,80	2,00	2,00	1,95	2,00	2,05	2,03	1,90	1,9250

The values are not satisfied either by a linear or by a quadratic equation.

Number of X:ta	Length of arm in microns									Total
	1	1,5	2	3	4	5	6	7	8	
2						1		2	1	4
1	51	73	99	60	59	59	20	14	19	454
0	9	7	1		1			4		22
Frequency	60	80	100	60	60	60	20	20	20	480
S(y)	51	73	99	60	59	61	20	18	21	462
$\bar{y}$	0,85	0,91	0,99	1,00	0,98	1,02	1,00	0,90	1,05	0,9625

The values are not satisfied either by a linear or by a quadratic equation.

i. *Pelobates fuscus.* Diplotene.

Number of X:ta	Length of chromosome in microns										Total
	4	5	6	7	13	15	16	17	19	20	
4										1	1
3						1			5	3	9
2	14	11	18	40	19	39	20	20	15	16	212
1	26	9	2		1						38
Frequency	40	20	20	40	20	40	20	20	20	20	260
S(y)	54	31	38	80	39	81	40	40	45	45	493
$\bar{y}$	1,35	1,55	1,90	2,00	1,95	2,03	2,00	2,00	2,25	2,25	1,8961

The values are not satisfied either by a linear or by a quadratic equation.

Number of X:ta	Length of arm in microns										Total
	1	2	3	4	5	6	9	10	11	13	
2							1	1	4	5	11
1	25	20	98	80	79	20	39	39	36	35	471
0	35		2		1						38
Frequency	60	20	100	80	80	20	40	40	40	40	520
S(y)	25	20	98	80	79	20	41	41	44	45	493
$\bar{y}$	0,42	1,00	0,98	1,00	0,99	1,00	1,03	1,03	1,10	1,13	0,9481

The values are not satisfied either by a linear or by a quadratic equation.

k. *Rana arvalis.* Diplotene.

Number of X:ta	Length of chromosome in microns									Total
	4	5	6	7	8	13	14	17	19	
3				1				1	1	3
2	13	19	38	18	19	59	20	19	19	224
1	7	1	2	1	1	1				13
Frequency	20	20	40	20	20	60	20	20	20	240
S(y)	33	39	78	40	39	119	40	41	41	470
$\bar{y}$	1,65	1,95	1,95	2,00	1,95	1,98	2,00	2,05	2,05	1,9583

The values are not satisfied either by a linear or by a quadratic equation.

Number of X:ta	Length of arm in microns										Total
	1	2	3	4	5	6	7	8	9	10	
2				1			1		1		3
1	34	19	98	56	59	40	39	60	19	40	464
0	6	1	2	3	1						13
Frequency	40	20	100	60	60	40	40	60	20	40	480
S(y)	34	19	98	58	59	40	41	60	21	40	470
$\bar{y}$	0,85	0,95	0,98	0,97	0,98	1,00	1,03	1,00	1,05	1,00	0,9792

The values are not satisfied either by a linear or by a quadratic equation.

l. *Rana temporaria.* Diplotene.

Number of X:ta	Length of chromosome in microns									Total
	3	4	5	6	11	12	13	15	18	
2	9	29	8	27	8	10	10	10	10	121
1	1	1	2	3	2					9
Frequency	10	30	10	30	10	10	10	10	10	130
S(y)	19	59	18	57	18	20	20	20	20	251
$\bar{y}$	1,90	1,97	1,80	1,90	1,80	2,00	2,00	2,00	2,00	1,9308

The values are not satisfied either by a linear or by a quadratic equation.

Number of X:ta	Length of arm in microns										Total
	1	2	3	4	5	6	7	8	9	10	
1	16	87	18	40	20	20	10	10	20	10	251
0	4	3	2								9
Frequency	20	90	20	40	20	20	10	10	20	10	260
S(y)	16	87	18	40	20	20	10	10	20	10	251
$\bar{y}$	0,80	0,97	0,90	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,9654

The values are not satisfied either by a linear or by a quadratic equation.

m. *Emys orbicularis.* Diakinesis. The 11 largest bivalents in every cell are analysed.

Number of X:ta	Chromosome pair No.											Total
	11	10	9	8	7	6	5	4	3	2	1	
3						1		1	1		4	7
2	7	8	7	5	5	5	7	7	6	7	4	68
1	1		1	3	3	2	1		1	1		13
Frequency	8	8	8	8	8	8	8	8	8	8	8	88
S(y)	15	16	15	13	13	15	15	17	16	15	20	170
$\bar{y}$	1,88	2,00	1,88	1,63	1,63	1,88	1,88	2,13	2,00	1,88	2,50	1,9318

As it was not possible to observe the mitotic lengths, the relation cannot be determined.

n. Anura. Diplotene.

Number of X:ta	Length of chromosome in microns																		Total
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
4																		1	1
3					1	2		1		4			1	1	2	1	6	3	22
2	54	192	174	160	78	76	19	77	28	101	128	79	49	39	58	28	34	16	1390
1	36	38	16	10	1	2	1	2	2	5	2	1				1			117
Frequency	90	230	190	170	80	80	20	80	30	110	130	80	50	40	60	30	40	20	1530
S(y)	144	422	364	330	160	160	39	159	58	219	258	159	101	81	122	60	86	45	2967
$\bar{y}$	1,60	1,83	1,92	1,94	2,00	2,00	1,95	1,99	1,93	1,99	1,98	1,99	2,02	2,03	2,03	2,00	2,15	2,25	1,9392

The values are not satisfied either by a linear or by a quadratic equation.

Number of X:ta	Length of arm in microns													Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
2				1	2	1	3	2	4	1	4	1	5	24
1	245	551	472	334	374	358	83	188	116	109	36	18	35	2919
0	75	19	8	5	4	1	4					1		117
Frequency	320	570	480	340	380	360	90	190	120	110	40	20	40	3060
S(y)	245	551	472	336	378	360	89	192	124	111	44	20	45	2967
$\bar{y}$	0,77	0,97	0,98	0,99	0,99	1,00	0,99	1,01	1,03	1,01	1,10	1,00	1,13	0,9696

The values are not satisfied either by a linear or by a quadratic equation.

of homologizing them from nucleus to nucleus. There is, however, nothing to indicate that this species deviates from the other Anura, except as regards the chromosome number (cf. Tables 2, 3, and 4). This is of interest, seeing that *Xenopus* belongs to that group of Anura which is considered to be most primitive (*v. infra*).

It appears from Table 5 and Graphs I—V that *in Protopterus and the Urodela the number of chiasmata is correlated to the chromosome length or arm length.* In *Protopterus* and *Triturus vulgaris* the regression seems to follow a linear equation, that is, the number of chiasmata is directly proportional to the chromosome or arm length. In *Triturus cristatus* and *Pleurodeles*, on the other hand, the regression seems to follow a quadratic equation, that is, the number of chiasmata rises with increasing chromosome length, but falls again in the very longest chromosomes or arms.

In Anura and Emys there is practically no correlation (Table 5 e—n). The number of chiasmata in the smallest chromosomes (shortest arms) rapidly rises to 2 per bivalent (1 per arm pair), and then remains practically unchanged, regardless of any increase in the chromosome or arm length.

These conditions are closely connected with the localization of the chiasmata. The diplotene pictures (Figs. 16—27; Plate II, Fig. 1) in fact indicate that the chiasmata are situated chiefly at the *distal* ends. This is clearly shown by the following analysis (Table 6).

TABLE 6. *Number of chiasmata within different chromosome regions. Average of five diplotene nuclei. Triturus vulgaris.* (*Cf. Fig. 39.*)

	Region I	Region II	Region III
	Distal end—5 μ from it	5—10 μ from distal end	10—15 μ from distal end
Number of arms within reg.	24	19	13
Total length of reg. in microns	115,5	85,5	38,0
Number of chiasmata within reg.(Total)	121,0	18,0	15,0
Number of chiasmata/nucleus within reg.	24,2	3,6	3,0
Number of chiasmata/arm and nucleus within reg.	1,0084	0,1895	0,2308
Number of chiasmata/micron within reg.	0,2096	0,0421	0,0792

The probability of this distribution of the chiasmata being due to chance is indicated by the following calculation:

	Reg. I	Reg. II	Reg. III	Total
Observed number of chiasmata	121	18	15	154
Expected number of chiasmata (e)	$\frac{115{,}5 \cdot 154}{239} = 74{,}4$	$\frac{85{,}5 \cdot 154}{239} = 55{,}1$	$\frac{38 \cdot 154}{239} = 24{,}5$	154
$\frac{(\text{Deviation})^2}{e}$	29,19	24,98	3,68	$\chi^2 = 57{,}85$

This value of χ^2 gives $P < 0{,}001$, whence *the chiasmata are not distributed by random but localized towards the ends of the chromosomes.*

The same result is shown by Table 7, where chromosome arms longer than 9 microns in *Triturus vulgaris* have been divided into regions, Reg. I comprising the distal end — 9 microns from it, Reg. II the remainder of the arm.

TABLE 7. *Number of chiasmata per micron in different regions of chromosome arms of 10 microns or more in length. Triturus vulgaris. 5 diplotene cells (cf. Fig. 39).*

Chromosome and arm	Region I												
	3L[1]	1L	7L	2L	1k[1]	5L	8L	6L	2k	4L	3k	4k	10L
Number of chiasmata	6	5	7	6	5	6	5	5	6	5	5	5	6
Length in microns	9	9	9	9	9	9	9	9	9	9	9	9	9
	Region II												
Number of chiasmata	3	3	3	2	0	2	2	2	0	3	0	0	1
Length in microns	6	5,5	5	5	5	4,5	4	4	4	3	2	2	1

An analysis of variance of the difference in the number of X:ta/micron between the two regions gives $0{,}01 > P > 0{,}001$.

From this it follows that in the arms which are 10 microns or more

[1] 3L; 1k = long arm of chromosome pair No. 3; short arm of chromosome pair No. 1, and so forth.

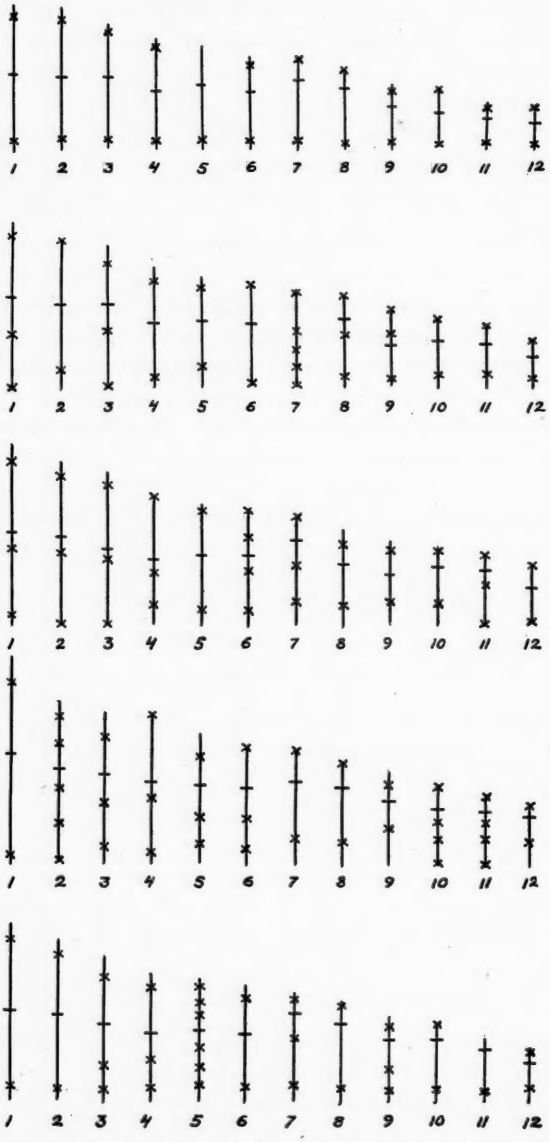


Fig. 39. Diagram of the localization of chiasmata in five diplotene nuclei of *Triturus vulgaris*. The chiasmata are marked by »×», the centromeres by »—». — 800 ×.

in length, *the number of chiasmata per micron is undoubtedly larger within the region distal end — 9 microns from it than from that point to the centromere.*

There are several possible explanations of these facts. Either this chiasma distribution is secondary and due to the terminalization, or else it is primary, and shows, more or less, the original chiasma distribution. In the latter case it may be due to marked interference round the centre mere or to the fact that the chromosomes are beginning to pair terminally and, for some reason, are unable to complete the pairing along their whole length.

That this localization of the chiasmata is partly due to the terminalization seems indubitable; on the other hand, the diplotene pictures (Figs. 16—27 and 39) show that this explanation does not suffice to settle the whole problem.

Thus, in the small chromosome pairs chiasmata are often found in a less terminal position, relatively speaking, than in the large pairs in the same nucleus, despite the fact that small chromosomes show a higher degree of terminalization than the large ones (DARLINGTON, 1937, p. 509 ff.).

A terminal localization of the chiasmata, one would think, might conceivably be due to interference round the centromere, though scarcely in such a high degree as here. No parallel cases have previously been reported. Moreover, if this supposition is correct, such interference should be about equally strong on both sides of the centromere.

An attempt has been made by the author to elucidate this matter by examining the chiasma distribution in chromosome pairs with one long and one short arm. In the existing material, the chromosome pairs best suited for the purpose are Nos. 5, 6, 7, and 8 in *Triturus vulgaris*, each of which has one arm longer, and the other shorter, than 10 microns. The results of such an investigation are summarized in Table 8, where a comparison has been made between region I = the short arm and region II = the proximal segment of the long arm 9 microns from the end to the centromere.

An analysis of variance of the number of chiasmata per micron in the short arm, as compared with that in the proximal part of the long one, gives $0{,}01 > P > 0{,}001$.

The difference in chiasma frequency will be brought out still more clearly if the short — though *relatively* long — arms of the chromosome pairs 5 and 6 are excluded, when $P < 0{,}001$.

Thus, between the regions referred to in Table 8, which are

TABLE 8. *Triturus vulgaris. Chromosomes 5, 6, 7, and 8. Chiasma frequency in different segments.*

Number of chiasmata	Region I				Region II			
	Chromosome No.				Chromosome No.			
	8	7	6	5	8	7	6	5
3	—	—	—	1	—	—	—	—
2	—	—	1	—	—	—	—	—
1	5	5	4	3	2	3	2	2
0	—	—	—	1	3	2	3	3
Frequency	5	5	5	5	5	5	5	5
Length in microns	4	4	9	9	4	4	4,5	5

practically of the same size, and situated at an equal distance from the centromere, on either side of it, there is a very considerable difference in chiasma frequency.

From this we can draw two important conclusions:

(1) *The chiasma frequency is not a function of the distance from the centromere, whence the peculiar localization of the chiasmata in Protopterus, Urodela, and Anura cannot be due to interference r o u n d the centromere.*

(2) *Pairing, with the formation of chiasmata, cannot proceed across the centromere.* In other words, *there is no positive correlation between chiasmata on one side of the centromere and the other.*

On the other hand, a negative correlation does not account for the peculiar localization of the chiasmata: in that case it would be very strange if chiasmata were almost always formed in the short arm. The lack of correlation in this respect between the two arms of a chromosome has previously been observed in *Drosophila* (MATHER, 1936), *Fritillaria* (BENNETT, 1938), and *Uvularia* (BARBER, 1941), whilst the reverse has been found in other species of *Fritillaria* (DARLINGTON, 1937; FRANKEL, 1940), as well as in *Culex* and *Dicraniomya* (PÄTAU, 1941).

The true explanation of the terminal localization of the chiasmata must, instead, be the initiation of the pairing at the ends of the chromosomes and its interruption before it has advanced some distance towards the centromere. Such interruption of the pairing has been interpreted by DARLINGTON (1937) as being due to a »time limit»; that is to say, the pairing is interrupted by the division of the chromosomes

at the beginning of diplotene. *This division must begin at the centromere and proceed towards the ends* (MATHER, 1936). This has been shown also by BOOST and LUDWIG (1939). The commencement of the division at the centromere has also been confirmed by the microscopical observations made by DARLINGTON (1937) in *Fritillaria*, where, however, the pairing also begins at the centromere. *This explains why, in these forms, pairing, and thus the formation of chiasmata, cannot proceed from the one arm of a chromosome across the centromere to the other arm* (*v. infra*).

If we compare these conditions with the previously known facts concerning other organisms, it will be found that they involve something new, though they can easily be interpreted in accordance with DARLINGTON's (1937) theories of meiosis. Distal localization of chiasmata has previously been quite unknown, with the exception of the case reported by KLINGSTEDT (1936) from *Chrysochraon dispar*. He explains this localization in the same way as has been done here. It should be observed, however, that KLINGSTEDT investigated only the metaphases, whence it cannot be regarded as established that this localization is primary and not caused by terminalization in *Chrysochraon*. There are indications of a distal chiasma localization also in *Tradescantia* (PÄTAU, 1940). Nor does this case either seem to have been studied with sufficient thoroughness. DARLINGTON (1940 a), however, adduces both *Chrysochraon* and *Tradescantia* as typical examples of a distal chiasma localization — in my view, on rather unsubstantial grounds.

The theory advanced here also covers the fact that such marked differences can occur as those between the species dealt with here, despite similarity in regard to the points at which pairing and division are initiated. I explain them as being due partly to a difference in time limit, i. e. the time between the commencement of the pairing and of the division, partly to differences in the length of the chromosomes. The most primitive conditions in this respect (cf. DARLINGTON, 1937, p. 295) seem to exist in *Protopterus*, where the time limit is so wide that the pairing is practically completed, and the chiasma frequency is thus a function of the arm length. In *Triturus cristatus* we find a modification of this: the long arms, owing to their less mobility, find less time to pair during the evidently tightened time limit. This applies particularly to the longest arm, which is further impeded in its movements by the nucleolus attached to it (cf. Scheme 1 and Graphs II and III). These difficulties of the long arms are somewhat lightened by the localization of the points at which pairing and division are initiated:

the long arms, even if they begin to pair later than the short ones and if the division begins simultaneously in all the chromosomes, thus have time to develop as many or more chiasmata than the smaller arms, as in a long arm the division has a longer distance to travel from the centromere towards the distal end before it reaches the paired part, than in a short one.

A further modification is found in *Triturus vulgaris*, where the differences in chiasma frequency between different arm lengths are still smaller, *i. e.* the time limit is still narrower (cf. Graphs IV and V). The difference in the degree of localization between *Triturus cristatus* and *vulgaris* has previously been pointed out by CALLAN (1942), but without figures or explanations.

The time limit seems to be most restricted in Anura, where almost all the arms except the very smallest develop one chiasma (cf. Table 5). Such variation as is found in the long arms shows that in favourable positions they can begin their pairing relatively early, and then find time to develop two chiasmata, whilst in particularly unfavourable positions they remain unpaired. This applies especially if they carry nucleoli, as, for example, the second largest arm (the largest chromosome) in *Hyla* (cf. Table 5 and Scheme 1). The same conditions as in Anura seem to exist in *Emys* (Table 5).

Besides these time-limit differences, the interference seems to be greater in Anura than in Urodela. This is indicated by the marked difference in the slope of the curves for the chromosome length: chiasma frequency between Urodela and Dipnoi, on the one hand, and Anura, on the other, even after cold treatment (p. 302). Cf. MATHER (1937, 1938 a).

The theory regarding the localization of chiasmata is strongly supported by the results which the present writer and other investigators have obtained in temperature experiments. Temperature effects which conduce to impede the pairing or to increase the rapidity of division also tend to reduce the chiasma frequency and *vice versa* (*v.* p. 293).

Another indication of the correctness of this view is given by the heterochromatic parts in Anura and Urodela — first discovered by CALLAN (1942) in *Triturus* and observed also by the present author —, which are unusually long and lie in the main centrically, that is, where chiasmata are seldom developed. Moreover, almost the whole proximal half of the nucleolus-carrying arm in *Triturus cristatus* is heterochromatic (cf. Table 5 and Scheme 1). These heterochromatic parts appear to have arisen in the same way as the heterochromatic sex chro-

mosomes and the heterochromatin in the chromosomes of *Drosophila* (MULLER, 1916; MATHER, 1936; DARLINGTON, 1937, p. 330; DARLINGTON and LA COUR, 1940, 1942; CALLAN, 1942). Such heterochromatic segments are still more marked in Anura. Though they have not been examined in detail, their distribution in *Rana esculenta* is indicated by Fig. 14. As will be seen, they lie in the main centrically. — In regard to the distribution of heterochromatic segments in different organisms in relation to the initiation of pairing and the localization of the chiasmata, the reader is referred to CALLAN (1942, p. 334, Table 1).

The conditions in Amphibia are particularly interesting, when compared with certain other well-studied organisms, *e. g. Uvularia* (BARBER, 1941). In *Uvularia* the pairing is preproximal, according to BARBER owing to the position of the chromosomes in the last premeiotic mitosis. In the pairing, chromosomes with terminal centromeres have an advantage over those with median centromeres, as the former must be more mobile. If the pairing is interrupted at an early stage, *e. g.* by high temperature (BARBER, *op. cit.*), this must result in proximally localized chiasmata. The distal parts of the chromosomes in *Uvularia*, however, apparently often pair before the intermediate parts. This too, according to BARBER, may be explained as a consequence of the localization in the nucleus, as, with the centromeres concentrated at the former telophase pole, the ends should be quite close to one another at the opposite pole. If the pairing is interrupted now, proximally and distally localized chiasmata will result. Normally, the pairing proceeds along the whole chromosome, whence the chiasma frequency will be a function of chromosome length.

In some respects the conditions in Anura are the direct opposite, in other ways a parallel, to those found in *Mecostethus* by WHITE (1936). There, too, the number of chiasmata is without relation to the chromosome length, and each chromosome pair has one chiasma; in *Mecostethus*, however, it is situated close to the centromere, where the pairing evidently begins. The similarity between Anura and *Mecostethus* is thus the strong localization of the chiasmata, whilst the dissimilarity consists in their position. *Fritillaria meleagris* resembles *Mecostethus* in this regard (DARLINGTON, 1937).

The localization of the chiasmata in *Fritillaria* (DARLINGTON, 1937), *Mecostethus* (WHITE, 1936), *Uvularia* (BARBER, 1941), as well as in Dipnoi, Urodela, Anura, *Emys*, and other forms — and thus to some extent the chiasma frequency —, thus depends mainly on a combination of the following factors:

(1) The point(s) where the pairing begins, the »contact point», DARLINGTON (1940 b). This point may be determined by the polarization (»bouquet»), as in the forms dealt with here, or by the position of the chromosomes in the last premeiotic mitosis, as in *Uvularia*, or by other factors.

(2) The greater liberty of movement in the small chromosomes and the ends of large ones, and possible impediments in the form of nucleoli or the like.

(3) The point(s) where the division begins. In the material hitherto examined, it seems to be proximal, but it is conceivable that deviations may occur.

(4) The time limit, *i. e.* the time before the division commences in each chromosome segment at the beginning of diplotene.

b. Abnormal chromosome numbers.

A curious case has been observed in *Pleurodeles* (Fig. 18). A diplotene cell was found to have thirteen bivalents instead of the normal twelve. All of them had a normal appearance and the chiasma frequency was likewise quite normal. This abnormality seems to be due to non-disjunction in a previous division. It was apparently the fifth or sixth pair that was thus present in duplicate. The non-formation of multivalents is in all probability due to the low chiasma frequency (*v.* the following section).

c. Triploids.

The triploids examined by the author consist (1) of one specimen of *Triturus vulgaris* and (2) four specimens of *Rana esculenta*. The *Triturus* specimen is the same as that dealt with by BÖÖK (1940, 1945). BÖÖK has kindly lent me the preparations and has allowed me to study his then unpublished results of his investigations of this triploid. Owing to the not quite satisfactory fixation (BOUIN-ALLEN), it was unfortunately found impossible to make a thorough study of the meiotic prophase in this specimen: the analysis was therefore to be confined to M^I. BÖÖK, in the papers referred to, has also made a number of observations in regard to the second division of meiosis. — The *Rana esculenta* specimens are from Bornholm, Denmark.

Triturus vulgaris. — As regards the frequency of the different configurations, it should be mentioned that the number of univalents in eleven out of twenty-three cells is less than might have been expected in view of the number of trivalents (BÖÖK, 1945). This has been ex-

plained by BÖÖK as being due to the occurrence of homologous segments in non-homologous chromosomes, *i. e.* to intrahaploid pairing. Pairing between these segments, however, has not been observed in the diploid. This, however, may be accounted for by competition in pairing (MÜNTZING, 1936; DARLINGTON, 1937).

The chiasma frequency in *Triturus vulgaris* 3n and 2n is shown in Tables 9 and 10. [The figures for the triploid from BÖÖK (1945); for the diploid the author's own figures.]

TABLE 9. *The chiasma frequency in triploid and diploid Triturus vulgaris. Metaphase.*

Number of chiasmata	Triploid (23 nuclei)				Diploid (20 nuclei)	
	Univ.	Biv.	Triv.	Tot. X:ta	Biv.	Tot. X:ta
0	122	—	—	—	—	—
1	—	64	—	64	23	23
2	—	85	125	420	199	398
3	—	—	11	33	18	54
Total	122	149	136	517	240	475
Total number of chromosomes.....	122	298	408	828	480	480
Number of chiasmata/nucleus			22,5		23,8	
Number of $^1/_2$ chiasmata/chromosome			1,25		1,98	
Number of chiasmata/configuration			1,81		1,98	

TABLE 10. *Comparison between triploid and diploid Triturus vulgaris in regard to number of chiasmata/configuration.*

	Configurations with				Number of nuclei
	1 X	2 X:ta	3 X:ta	Total	
Diploid	23	199	18	240	20
Triploid	64	210	11	285	23
Total	87	409	29	525	

A χ^2 analysis of the number of chiasmata/configuration in the triploid compared with the diploid gives $\chi^2 = 7{,}80$ ($P = 0{,}02$).

An analysis of variance of the difference between the number of $^1/_2$ X:ta/chromosome in the triploid and diploid gives $P < 0{,}001$.

Rana esculenta. — The chiasma frequency in 2n and 3n of this species is shown by Tables 11 and 12. (Cf. Figs. 34 and 38.)

TABLE 11. *The chiasma frequency in triploid and diploid Rana esculenta. Diplotene.*

Number of chiasmata	Triploid (8 nuclei)				Diploid (20 nuclei)	
	Univ.	Biv.	Triv.	Tot. X:ta	Biv.	Tot. X:ta
0	100	—	—	—	—	—
1	—	3	—	3	20	20
2	—	94	6	200	237	474
3	—	—	—	—	3	9
Total	100	97	6	203	260	503
Total number of chromosomes.....	100	194	18	312	260	520
Number of chiasmata/nucleus			25,38		25,15	
Number of $^1/_2$ chiasmata/chromosome			1,30		1,93	
Number of chiasmata/configuration			1,92		1,93	

TABLE 12. *Comparison between triploid and diploid Rana esculenta in regard to number of chiasmata/configuration.*

	1 X	2 X:ta	3 X:ta	Total	Number of nuclei
Diploid	20	237	3	503	20
Triploid	3	100	—	203	8
Total	23	337	3	706	—

$\chi^2 = 2{,}04$ $(0{,}20 > P > 0{,}10)$.

An analysis of variance of the difference in the number of $^1/_2$ X:ta per chromosome between the diploid and the triploid gives $P < 0{,}001$.

From Tables 9—12 it appears that the number of $^1/_2$ X:ta per chromosome is significantly different in the diploids and the triploids. The distribution of the chiasmata per configuration differs in the triploid and diploid as regards *Triturus*, but not in *Rana*. The following reasons may be adduced in explanation of these facts.

Since in a triploid at zygotene a third of the set must remain unpaired, it follows that, with the same interference in the triploid and diploid, the number of chiasmata per nucleus must be the same in both.

From this it may be inferred that, as the number of chromosomes, but not of chiasmata, in the triploid has been increased by a third, the number of half-chiasmata per chromosome in the triploid must be two-thirds of that in the diploid. Furthermore, the number of configurations (bivalents + trivalents) in normal cases must be the same in the triploid and diploid, whence also the number of chiasmata per configuration must be the same.

In *Triturus*, as shown above, the number of configurations in the triploid is larger than might have been expected, owing to the decrease in the number of univalents due to intrahaploid pairing. As the segments which pair in this intrahaploid pairing must be comparatively short (evidently, they hardly ever pair in the diploid), the configurations produced by intrahaploid pairing should never have more than one chiasma. Thus the reduction in the number of chiasmata per configuration in the triploid of *Triturus vulgaris* must, in some measure, be attributed to intrahaploid pairing. This explanation, however, does not cover the whole decrease, as is evident from the fact that the number of chiasmata per cell is lower in the triploid than in the diploid.

In *Rana esculenta*, where intrahaploid pairing does not occur, there is a very good correspondence between the triploid and the diploid as regards the number of chiasmata per configuration.

The number of half-chiasmata per chromosome in both these triploids corresponds fairly well with two-thirds of the number in the respective diploids (*Triturus vulgaris* $1{,}25$—$1{,}32$; *Rana esculenta* $1{,}30$—$1{,}29$).

Now, how far do these observations correspond with those made in regard to other organisms? In the said respects, only 5 forms have been previously dealt with, namely *Allium Schoenoprasum* (LEVAN, 1936), *Fritillaria latifolia major* (DARLINGTON, 1940 b), *Hyacinthus* (STONE and MATHER, 1932), *Tulipa* (DARLINGTON and MATHER, 1932), and *Solanum lycopersicum* (UPCOTT, 1935). The conditions in these forms are shown in Table 13.

In *Allium*, *Fritillaria*, *Hyacinthus*, and *Tulipa*, as will be seen, the chiasma frequency is practically the same in the diploid and triploid (or the triploid has higher values), whereas *Solanum*, *Rana*, and *Triturus* show a chiasma frequency in the triploid corresponding rather well with two-thirds of that in the diploid.

In his study of triploid *Fritillaria latifolia*, DARLINGTON (1940 b) has modified and elaborated his earlier views (1937, p. 551) regarding the causes of these differences between diploids and triploids. He is

TABLE 13. *Number of $^1/_2$ X:ta/chromosome in triploid forms, as compared with the closely related diploids.*

Species	2n	3n	2/3 2n
Allium Schoenoprasum	2,8	2,7	1,9
Fritillaria latifolia major	2,3	2,8	1,5
Hyacinthus	2,7	2,6	1,8
Tulipa	2,1	2,1	1,4
Solanum lycopersicum	1,7	1,2	1,1
Triturus vulgaris	1,98	1,25	1,32
Rana esculenta	1,93	1,30	1,29

now of the opinion that the high chiasma frequency in triploids, as compared with what we should have reason to expect in view of the conditions in diploids, seeing that the length of chromosome which, theoretically speaking, can pair and form chiasmata should be the same in both, must be due to the greater number of contact points in the triploid, which prevent the uncoiling of the intercalary segments and thus augment the coiling tension.

This explanation of the differences between diploids and triploids is borne out by the author's results. The narrow time limit and distal initiation of pairing which in the diploid give rise to the remarkably low chiasma frequency, relatively to the length of chromosome, in *Triturus vulgaris* and *Rana esculenta* entail the consequence that in the triploid trivalents are rare, especially so in *Rana* (Fig. 38, Tables 9 and 11). Changes of partner therefore seldom occur, whence no increase in the number of $^1/_2$ chiasmata per chromosome, as compared with the theoretically expected two-thirds of that of the diploid, is found in these species. Another reason for this is that so many chromosomes in these species have median, or almost median, centromeres (cf. DARLINGTON, 1940 b).

A form which seems, to some extent, to be a parallel to the triploids of *Triturus vulgaris* and *Rana esculenta*, is *Paris hexaphylla 3n*, studied by HAGA (1937). This species corresponds most closely to *Rana esculenta*. *Paris* likewise has extremely few trivalents, evidently owing to the shortness of the paired region. However, the chiasmata in *Paris* are situated proximally, whilst in *Rana*, as shown above, they have a distal localization.

In this connection, however, some importance may be attached to the fact that the triploids of *Solanum*, *Rana*, and *Triturus* were the first triploid generation, whereas the *Hyacinthus*, *Tulipa*, and *Fritillaria* specimens are derived from ancient triploid clones. The great variation also between different diploid clones of *Tulipa* and *Hyacinthus* (DARLINGTON and MATHER, 1932; STONE and MATHER, 1932) indicates that genetic control of the chiasma frequency may play some part here. Some of the differences between the diploids and triploids in these species may possibly be due to genetic variations between different clones.

II. EXPERIMENTS.

A. TEMPERATURE TESTS.

a. The connection between chiasma frequency and temperature.

Previous literature.

It has long been known that the cell divisions and phenomena connected with them may be affected by various external and internal factors. That temperature plays an important part in this respect has long been pointed out by a large number of investigators (see HEILBORN, 1930; SAX, 1935, 1937; OEHLKERS, 1935, 1936, for literature).

Most of them have merely observed the effects of abnormally high or low temperature and found them to consist in various disturbances of meiosis. Some of these disturbances must undoubtedly be attributed to defects in the spindle, etc., and may therefore be set aside here. Thorough investigations of the changes in chiasma frequency in complete temperature series have been made only by WHITE (1934) and POLIAKOWA (1940), both of whom find that the chiasma frequency is comparatively low at a temperature normal for the organism in question, but increases when the temperature is raised or lowered in a certain degree (*v.* Graphs VI and IX, from WHITE and POLIAKOWA); and that, on a further rise or fall of the temperature towards the lethal points, the chiasma frequency is again reduced. — This last-mentioned reduction, however, has not been observed in all the organisms investigated. It is missing at high temperatures in WHITE's material, presumably because it does not occur until lethal temperatures have been reached. Nor has the increase in chiasma frequency on a rise of temperature been observed in *Schistocerca*. WHITE (1934, p. 209) hazards the ex-

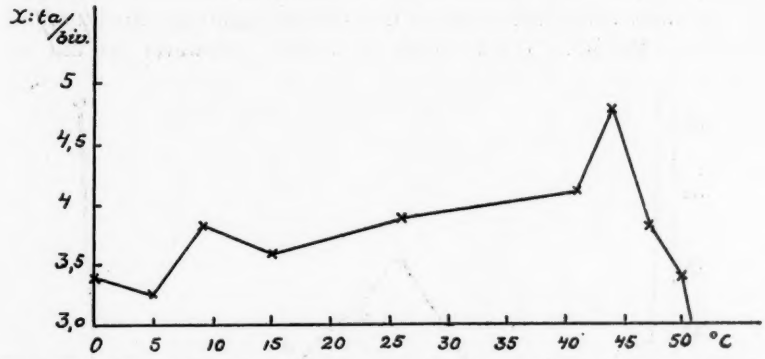
planation that such an increase probably does not occur before the temperature has exceeded 45° centigrade (the highest temperature to which he had subjected *Schistocerca*).

A corresponding curve results from PLOUGH's (1917) finding that in *Drosophila* the crossing-over frequency depends on the temperature (Graph VII, after PLOUGH). Here, too, the reduction in chiasma frequency at very high temperature is missing. The explanation seems to be that this part of the curve eludes observation, as at this temperature the flies have become sterile.

Now, what are the factors primarily concerned in the variations of chiasma frequency on changes of temperature? BORGENSTAM (1922) is of the opinion that the primary effect of variations in temperature on the meiosis is to bring about changes of viscosity, and that these changes afterwards entail abnormities in the divisions. WHITE (1934) takes the same line, but deplores the absence of any investigation of the dependence of the plasma viscosity on the temperature. NAKAMURA (1936, p. 175) points out, however, that the curve published by HEILBRUNN (1928) on the connection between plasma viscosity and temperature, in view of its steep gradients for the rises in viscosity at abnormally high or low temperatures, shows a certain connection with the abnormalities which these temperatures produce in the cell divisions. NAKAMURA (1936, p. 180) adds: ». . . such abnormal change of viscosity may be associated with the unbalance of the energies which control the mechanism of meiosis». Though POLIAKOWA (1940) is likewise of the opinion that the effect of variations in temperature on the meiosis is primarily due to changes of viscosity, she considers that the principal factor is the viscosity of the chromosomes themselves, a higher viscosity in them entailing a lower chiasma frequency and *vice versa*. She does not, however, adduce any evidence in support of this view, nor does she back it up by parallels with the results of other investigators.

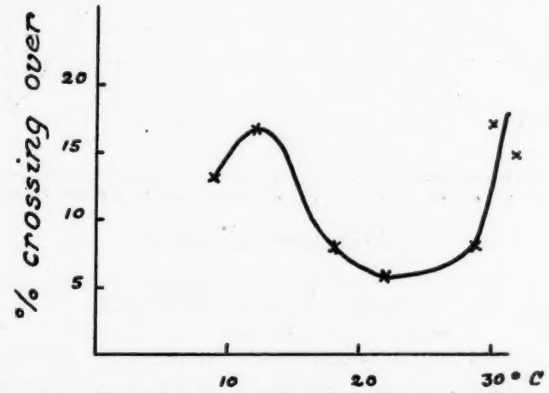
If, however, we compare the curve published by HEILBRUNN (1928), showing how, in the egg of *Cumingia*, the viscosity changes according to variations in temperature, with the curves published by WHITE (1934), POLIAKOWA (1940), and PLOUGH (1917), showing the changes in chiasma or crossing-over frequency at different temperatures (see Graphs VI—IX), we shall be immediately struck by their correspondence. They distinctly indicate that the chiasma frequency is negatively correlated with the viscosity of the plasma, as this is shown by HEILBRUNN's Figure. If a similar curve is applicable also to the viscosity in the plasma of Insecta and *Allium*, it is difficult to avoid the conclusion

that a negative correlation actually exists. On close examination of the curves, it will be found that the viscosity is rather high at the optimal temperature for the organism in question, the chiasma frequency being



Graph VI. Chiasma frequency in *Allium* at various temperatures. From POLIAKOWA (1940).

consequently rather low. If the temperature is raised or lowered, the viscosity is reduced, with a consequent increase of the chiasma frequency, until, at the semilethal high or low temperatures, a sharp rise

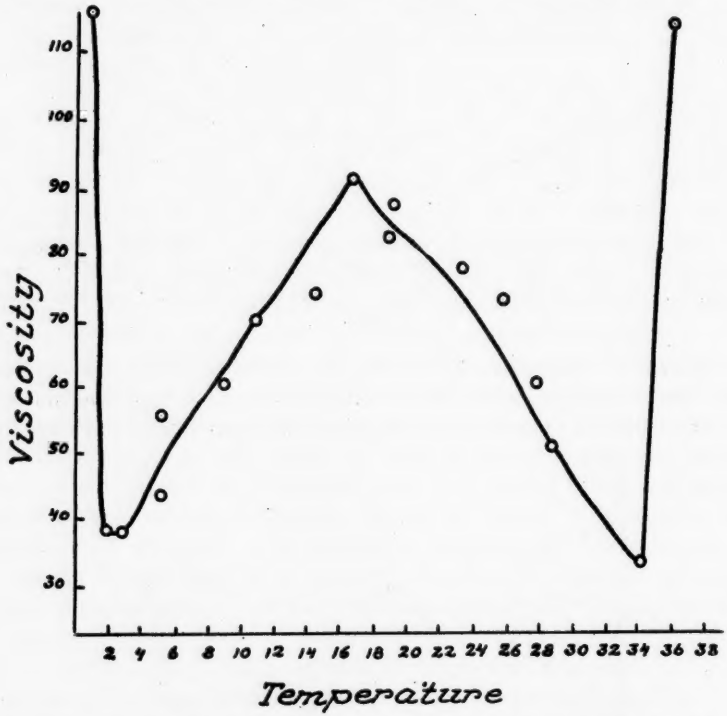


Graph VII. Crossing-over frequency in *Drosophila melanogaster* at various temperatures. From PLOUGH (1917).

of the viscosity occurs, with a resulting reduction of the chiasma frequency. This part of the chiasma frequency curves at high temperature is, however, missing in PLOUGH's and WHITE's material (*vide supra*).

In POLIAKOWA's study of *Allium*, however, this part of the curve is shown with great clearness. A similar reduction of the chiasma frequency is indicated also by *e. g.* HEILBORN's (1930), SAX's (1935, 1937), STRAUB's (1936), MATSUURA's (1937), and BARBER's (1941) results.

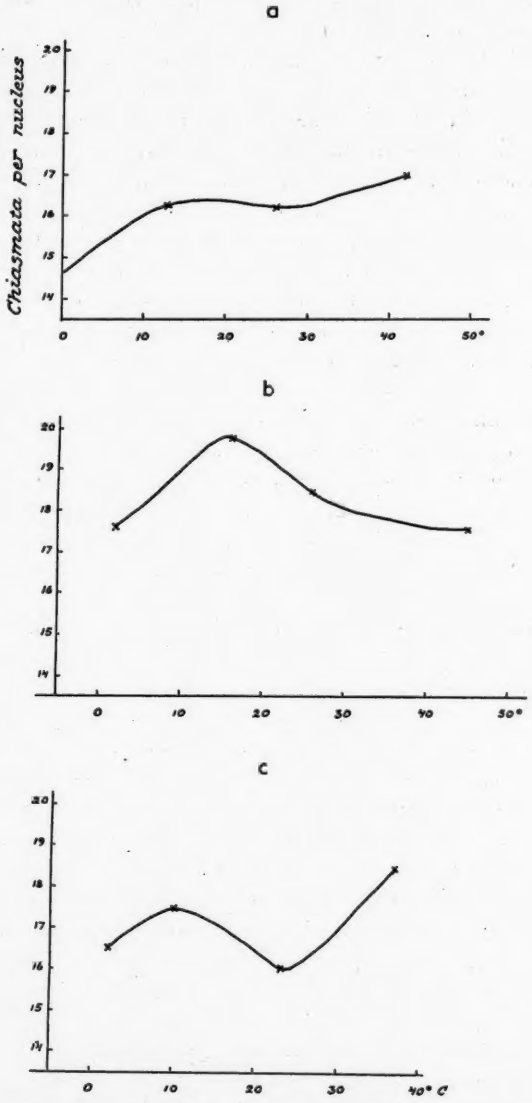
The only earlier investigation that cannot readily be fitted into this scheme is BRYDEN's (1935) study on *Rattus*. However, as this in-



Graph VIII. The relation between temperature and viscosity of the plasma of the egg of *Cumingia*. From HEILBRUNN (1928).

vestigator's figures are too small to give statistically reliable material, and as the effect of external temperature on a mammal with a constant body temperature can scarcely be so marked as to influence the chiasma frequency, his results need not be taken into account here.

Whether OEHLKERS's (1936) opinion that the chiasma frequency is more affected by sudden changes in temperature than by a constant low temperature can be reconciled with my own views, as set forth



Graph IX. Chiasma frequency at various temperatures. From WHITE (1934). a, *Locusta migratoria*; b, *Schistocerca gregaria*; c, *Stenobothrus parallelus*.

here, cannot be judged until the effect of such changes on viscosity, etc. has been investigated.

An additional fact which argues strongly in favour of the view that changes of viscosity are the primary effect of temperature is SAKAMURA's (1916) discovery that other factors which affect viscosity, such as chloral hydrate, influence the meiosis much in the same way as abnormal temperatures. Cf. also SHIGENAGA (1937).

Thus I consider it to be proved, or at any rate extremely probable, that the effect of temperature on chiasma frequency is not direct, but secondary to other factors, the most important of which, in all probability, are changes of viscosity. At least two alternatives are conceivable here. The determining factor is either the viscosity in the chromosomes themselves (their »rigidity») or else the viscosity of the plasma.

Certain indications point distinctly to the correctness of the last-mentioned supposition. The first-mentioned alternative is at variance with the fact that, as WHITE (1934) has found, the small chromosomes are not affected by variations in temperature. BARBER (1941) likewise observed that the small chromosomes were less affected, in regard to chiasma frequency, by temperature changes. If the rigidity of the chromosomes themselves were the determining factor — an altered rigidity would entail altered relational coiling at pachytene, with a consequently altered interference — this should, of course, apply in equal degree to *all* chromosomes irrespective of their length. The view that the viscosity of the chromosomes is the principal factor is also at variance with OEHLKERS's (1935) observations on *Oenothera* hybrids, where the same genome (*flavens* × ^{h}*Hookeri*) reacts differently in the plasma of *suaveolens* and in that of *Hookeri*. It may further be mentioned that STRAUB (1936, p. 51) — though unfortunately in an unduly small material — was unable to detect any differences in the density of relational coiling in *Campanula* after different temperature tests. MATHER's examination (1937, 1938 a) of the slope of the curve chiasma frequency : chromosome length in WHITE's material shows clearly that the interference is scarcely affected by variations in temperature.

The balance of probability is thus in favour of the second-mentioned alternative, namely that the dependence of the chiasma frequency on temperature is ultimately due to changes in the viscosity of the *plasma*. If this viscosity is increased, the chromosomes will have greater resistance to overcome in their conjugation, and this resistance will be pro-

portional to their size. Conversely, a reduction in the viscosity of the plasma should give the chromosomes, particularly the larger ones, greater facilities for finding one another and pairing completely. This is in correspondence with WHITE's (1934) and BARBER's (1941) results.

OEHLKERS's above mentioned findings in *Oenothera* hybrids are easy to explain by different sensibility to temperature changes in the plasma of different species.

It should be pointed out that, when the general contour of the curves for different organisms shows considerable similarity, it is quite justifiable to speak of a correspondence between them, even if the peaks and throughs lie at different temperatures. In the nature of things, organisms which normally live at a high temperature must have their optimum at a higher temperature than those which live at a lower one: the whole curve can thus be shifted in a corresponding direction. This in fact is strikingly shown by WHITE's curves (Graph IX). *Stenobothrus*, which is a European form, has a low chiasma frequency at about 20° centigrade, whereas the tropical forms, *Locusta migratoria* and *Schistocerca gregaria*, have corresponding points on the curves at 30° or over. *Drosophila*'s crossing-over minimum lies at 20—25° centigrade, which corresponds with the optimal temperature of that fly (PLOUGH, 1917). HEILBORN's (1930) observations on different varieties of apples are in good conformity with this, as also STRAUB's (1936) observation that *Campanula persicifolia*, which has its habitats in temperate climates, is much more sensitive to heat in this respect than the tropical species *Rheo discolor*.

Own results.

That the changes of the plasma viscosity with the temperature do not always suffice to explain the whole problem of the dependence of the chiasma frequency on temperature is, however, shown by my own investigations. They were made on *Triturus cristatus* and *Bufo calamita*. The animals were placed in glass jars with a wire-netting lid and containing an ample amount of moist moss, these jars were then placed in an incubator at + 26° or in a refrigerator at + 4° or + 6° centigrade. The temperature tests proceeded for 48 hours, whereupon the heat-treated animals were decapitated and their testes fixed. The jars with the cold-treated animals, on the other hand, were allowed to stand for two hours (in certain cases merely one) at room temperature, + 16°, in order to permit the resumption of the division processes, whereupon the testes were treated in the usual manner. The results are shown in Table 14 and Graphs X—XII.

TABLE 14. *Chiasma frequency, at different temperatures, in Triturus cristatus and Bufo calamita, and the relation between number of chiasmata and length of chromosome or chromosome arm.*

Triturus cristatus. + 6° C.

Number of X:ta	Chromosome length in microns											Total
	10	12	13	15	16	17	21	22	24	25	30	
6	—	—	—	—	—	—	1	—	1	1	—	3
5	—	—	—	—	—	1	3	3	3	3	—	13
4	—	1	3	5	3	5	3	3	3	1	5	32
3	3	10	4	2	4	1	—	1	—	2	2	29
2	4	3	—	—	—	—	—	—	—	—	—	7
Frequency	7	14	7	7	7	7	7	7	7	7	7	84
S(y)	17	40	24	26	24	28	33	30	33	31	26	312
$\bar{y}$	2,43	2,86	3,43	3,71	3,43	4,00	4,71	4,29	4,71	4,43	3,71	3,7143

Equation of the regression line. $Y = 0{,}5562x - 0{,}0122x^2 - 1{,}8722$. Cf. Graph X.

Number of X:ta	Length of arm in microns													Total
	3	4	5	6	7	8	9	10	11	12	13	14	20	
3	—	—	—	—	1	—	1	1	7	2	7	4	—	23
2	—	—	3	10	24	12	5	13	14	4	6	3	4	98
1	7	7	4	11	10	2	1	—	—	1	1	—	3	47
Frequency	7	7	7	21	35	14	7	14	21	7	14	7	7	168
S(y)	7	7	10	31	61	26	14	29	49	15	34	18	11	312
$\bar{y}$	1,00	1,00	1,43	1,48	1,74	1,86	2,00	2,07	2,33	2,14	2,43	2,57	1,57	1,8571

$Y = 0{,}3888x - 0{,}0146x^2 - 0{,}2553$. Cf. Graph XI.

Triturus cristatus. + 26° C.

Number of X:ta	Chromosome length in microns											Total
	10	12	13	15	16	17	21	22	24	25	30	
5	—	—	—	—	—	—	1	—	—	—	—	1
4	—	1	1	—	—	2	—	2	2	—	—	8
3	—	5	3	2	3	3	4	4	2	5	3	34
2	6	6	2	4	3	1	1	—	2	1	3	29
Frequency	6	12	6	6	6	6	6	6	6	6	6	72
S(y)	12	31	17	14	15	19	19	20	18	17	15	197
$\bar{y}$	2,00	2,58	2,83	2,33	2,50	3,17	3,17	3,33	3,00	2,83	2,50	2,7361

$Y = 0{,}2711x - 0{,}0064x^2 + 0{,}1761$. Cf. Graph X.

Number of X:ta	Length of arm in microns													Total
	3	4	5	6	7	8	9	10	11	12	13	14	20	
3	—	—	—	—	—	—	—	—	—	—	—	1	—	1
2	—	—	1	4	10	5	4	4	11	2	6	2	2	51
1	6	6	5	14	20	7	2	8	7	4	6	3	4	92
Frequency	6	6	6	18	30	12	6	12	18	6	12	6	6	144
S(y)	6	6	7	22	40	17	11	16	29	8	18	10	8	197
$\bar{y}$	1,00	1,00	1,17	1,22	1,33	1,42	1,67	1,33	1,61	1,33	1,50	1,67	1,33	1,3681

$Y = 0{,}5788 + 0{,}1437x - 0{,}0053x^2$. Cf. Graph XI.

Bufo calamita. + 4° C.

Number of X:ta	Length of arm in microns							Total
	1	2	3	4	5	6	7	
2	—	—	—	—	1	4	1	6
1	19	50	40	10	39	46	9	213
0	1	—	—	—	—	—	—	1
Frequency	20	50	40	10	40	50	10	220
S(y)	19	50	40	10	41	54	11	225
$\bar{y}$	0,95	1,00	1,00	1,00	1,03	1,08	1,10	1,0227

$Y = 0{,}0209x + 0{,}9420$. Cf. Graph XII.

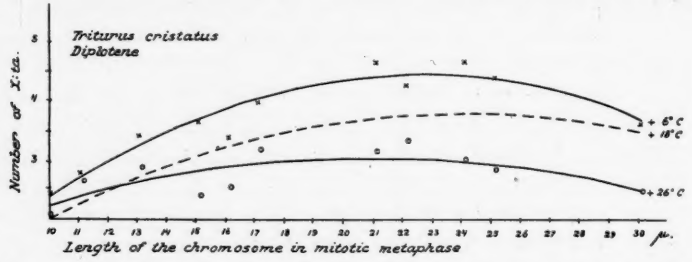
Bufo calamita. + 26° C.

Number of X:ta	Length of arm in microns							Total
	1	2	3	4	5	6	7	
1	11	32	27	7	28	35	7	147
0	3	3	1	—	—	—	—	7
Frequency	14	35	28	7	28	35	7	154
S(y)	11	32	27	7	28	35	7	147
$\bar{y}$	0,79	0,91	0,94	1,00	1,00	1,00	1,00	0,9500

The values are not satisfied either by a linear or by a quadratic equation. Cf. Graph XII.

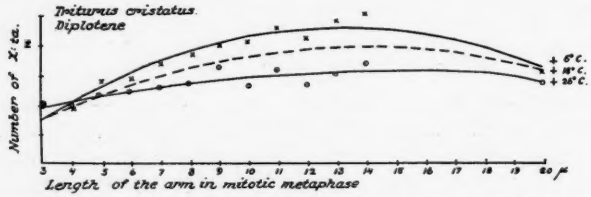
As the Table and Graphs show, the general effect of *low* temperature is to increase the chiasma frequency. It should be particularly noted, however, that in *Triturus cristatus* the smallest chromosomes and

arms are much less affected than the middle-sized elements. The very largest chromosome (and arm) deviates from the others, as also at



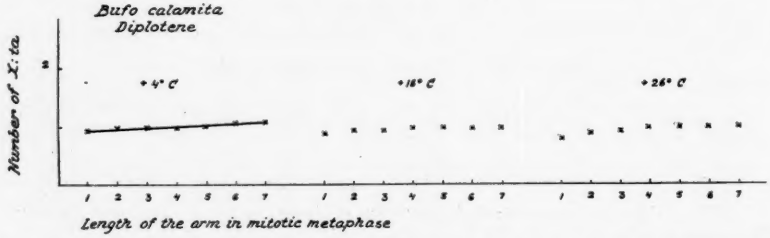
Graph X. Chiasma frequency of the chromosomes of *Triturus cristatus* at different temperatures. (Cf. Table 14.)

normal temperature, owing to the additional onus of the nucleolus. In *Bufo calamita* — in contradistinction from the conditions at normal



Graph XI. Chiasma frequency of the chromosome arms of *Triturus cristatus* at different temperatures. (Cf. Table 14.)

temperature — a direct proportion between arm length and chiasma frequency is observed at low temperature.



Graph XII. Chiasma frequency of the chromosome arms of *Bufo calamita* at different temperatures. (Cf. Table 14.)

In these animals the general effect of *high* temperature is to reduce the chiasma frequency. As regards *Triturus*, the marked flattening-out

of the curve indicates that the chiasma frequency has been reduced to about 1,5 per arm. Here, too, the small chromosomes of this newt are scarcely affected by the temperature. In *Bufo calamita*, on the other hand, high temperature seems to have no perceptible effect on the large chromosomes, whereas it evidently tends to reduce the chiasma frequency in the small ones.

In the material treated here, all the differences in chiasma frequency due to the temperature are statistically significant. An analysis of variation of the number of chiasmata in *Triturus vulgaris* at $+ 6°$, $+ 16°$ and $+ 26°$ centigrade gives $P < 0{,}001$; in *Bufo calamita* $P < 0{,}001$.

These results may appear to conflict with the preceding statements regarding the connection between chiasma frequency and temperature. This applies particularly to the findings at $+ 26°$, where, according to the previously cited results, we should have expected that the chiasma frequency, instead of a reduction, would have shown an increase.

The explanation, I consider, must be sought especially in the effect of temperature on the physiological processes which take place during the chromosome division at the end of pachytene. It is scarcely probable that in these organisms the marked premortal increase of viscosity which HEILBRUNN (1928) found in *Cumingia* at 34° should set in at 26°, as *Bufo* and *Triturus* can stand considerably higher temperatures. It is in fact generally known that physiological processes are affected, in regard to rapidity, by temperature [see BĚLEHRÁDEK (1935) for the literature]. It is thus plausible to suppose that the above-mentioned processes at the end of pachytene must likewise be dependent on temperature, *i. e.* that their rapidity increases with rising temperature.

An indication of this is DARLINGTON and LA COUR's (1940) discovery that very low temperature impedes the gene division. As even at normal temperature the time limit for the completion of the pairing is rather restricted, the effect of a rise in temperature will depend on the following circumstances. If the reduction of viscosity is very marked relatively to the increase in the rapidity of division, the result should be an extension of the time limit, with a resulting increase in chiasma frequency. If, on the other hand, the *division* is accelerated by the temperature in a greater degree than the pairing, the time limit will be narrowed and the chiasma frequency will be correspondingly reduced.

It is this latter process that apparently takes place in Amphibians. A similar view seems to be taken by STRAUB (1936, p. 51): »Es liegt nahe, anzunehmen, dass *jene Kraft, die in der Normalprophase die beiden Partner trennt, in anormalen Prophasen früher einsetzt*».

STRAUB also shows that the degree of terminalization is increased by low temperature and reduced by high temperature, probably because the latter tends to accelerate the cell division, so that the chromosomes do not find time for the terminalization of their chiasmata (cf. DARLINGTON, 1937, p. 512). Similar results are obtained by the present writer.

MATSUURA (1937) is likewise of the opinion that the meiosis is accelerated by heat. Another indication pointing in the same direction is HUSKINS and NEWCOMBE's (1941) finding that high temperature tends to diminish coiling: this is perhaps because the division of the cell proceeds so rapidly that the chromosomes do not find time for complete spiralization.

That such a restriction of the time limit should actually entail the results just mentioned seems rather probable on a comparison between BARBER's (1941) results and mine. In BARBER's experiments the temperature was so high that an increase in viscosity must have occurred. This is indicated by the fact that the small chromosomes had a higher chiasma frequency than the large ones. — Owing to their greater mobility, they are not affected so much as the large ones by the resistance of the plasma to their movements. — In my own material, on the other hand, an equalization sets in at 26°, so that in *Triturus* almost all the chromosomes find time to pair along a stretch sufficiently long for the development of one or at most two chiasmata. At normal or lowered temperature, on the other hand, the pairing can continue, so that in the chromosomes which have the requisite length additional chiasmata are developed.

Some importance must be attached also to the place at which pairing is initiated. In *Uvularia* (BARBER, 1941) it is usually preproximal, in Anura and Urodela predistal. For this reason, the long arms in these Amphibians, despite greater mechanical resistance, should have time to pair to about the same extent as the small ones, before the division beginning at the centromere (DARLINGTON, 1937; MATHER, 1937, 1938 a) reaches the paired parts, even if the division after a rise of temperature sets in earlier. In *Uvularia* the pairing usually begins at the centromere, whence the large chromosomes — which are more impeded than the small ones by the plasma viscosity which has been increased by the great rise in temperature — find time to pair merely for a short distance, or not at all, whereas the small ones, being more mobile, show a higher chiasma frequency.

In *Bufo* we find the same conditions as in *Triturus*, though, owing

to the normally lower chiasma frequency and smaller size of chromosome, still more accentuated. Here the short arms often do not find time to pair at all before division, whereas in the longer ones the chiasma frequency is reduced to 1 per arm at 26°.

It is thus evident that the effect of variations in temperature on the chiasma frequency is of a very complex nature, affecting it in at least two different ways. Firstly, they change the viscosity of the plasma, and thus also the rate of motion of the chromosomes. Secondly, they influence the speed of the reactions which bring about the division of the chromosomes at the end of pachytene and interrupt the pairing. Generally speaking, the more important of these twofold effects is the change of the plasma viscosity. As found by POLIAKOWA (1940) and WHITE (1934), such changes in response to varying temperatures, as a rule, produce a chiasma frequency curve of M-shape. According to physiological and chemical laws, the speed of the division should be proportional to the degree of temperature (BĚLEHRÁDEK, 1935). *The interplay between these two factors as well as the position of the points where the pairing and the division have been initiated determine the time limit for pairing and development of chiasmata.*

Certain other factors may possibly be involved, such as the fact that the volume of the nuclei changes with the temperature in accordance with a U-shaped curve (HARTMANN, 1919). HARTMANN's values for these changes are rather large. Thus the nuclear volume in *Pisum* at 8,5° and 37° centigrade is more than three times as large as at 31°. This must obviously affect the time available for the chromosome pairing.

MATHER (1938 b) points out that in *Drosophila* the effect of variations in temperature on the chiasma frequency must be attributed particularly to the heterochromatin. This, however, does not conflict with the views set forth here. The heterochromatin often shows a spiralization, etc. divergent from that of the euchromatin, and it is thus reasonable to suppose that the time limit for division may be correlated to the degree of spiralization, *i. e.* the time limit of the heterochromatin may be different from that of the euchromatin, being apparently more restricted in *Drosophila.*

It is by no means my intention to assert that the problem as to the effect of temperature on the chiasma frequency has thus been definitively solved. In the preceding paragraphs, however, I have endeavoured to show that light can be thrown on certain of these problems on the basis of DARLINGTON's theories regarding the zygotene

pairing and the time limit, as well as of the known facts concerning the effect of temperature changes on various physiological conditions in other organisms.

b. Terminalization of the chiasmata.

In addition to what has been stated in the preceding section in regard to the effect of temperature variations on the frequency and distribution of the chiasmata, a number of other observations have been made regarding the effect of different temperatures on *Triturus* and *Bufo*.

The degree of terminalization seems to diminish as the temperature rises (Table 15).

TABLE 15. *Terminalization coefficients at different temperatures. Triturus cristatus. Diplotene.*

	6° C	16° C	26° C
	0,35	0,28	0,20
	0,36	0,19	0,12
	0,22	0,15	0,17
	0,36	0,24	0,14
	0,29	0,09	0,16
	0,39	0,16	0,13
	0,33	0,16	—
	—	0,19	—
	—	0,15	—
	—	0,26	—
Mean =	0,33	0,19	0,15
n =	7 nuclei	10 nuclei	6 nuclei

An analysis of variation gives $P < 0{,}001$, whence the differences in the terminalization coefficients at different temperatures are statistically significant.

The explanation seems to lie in the effect of temperature on the meiosis, which is accelerated by heat (MATSUURA, 1937), but retarded by cold (BARBER, 1941; DARLINGTON and LA COUR, 1940). For this reason, high temperature does not give the chromosomes time for the terminalization of their chiasmata, whereas low temperature permits it to continue: this is explicable on the basis of DARLINGTON's electrostatic theory (DARLINGTON, 1937). Similar results, though on too small a material to admit of reliable conclusions, have been obtained by STRAUB (1936).

c. Spiralization.

Similar conditions seem to exist as regards spiralization. At high temperature it is lower, at low temperature stronger (Fig. 14) than in corresponding stages at normal temperature. The reason is apparently the same as that noted above in regard to terminalization. For further particulars see DARLINGTON and LA COUR (1942).

d. Inactivation of the spindle and inhibition of division.

Low temperature also tends to inactivate the spindle, thus giving rise to polyploidy (BARBER, 1939; FANKHAUSER and GRIFFITHS, 1939; GRIFFITHS, 1940, 1941; BÖÖK, 1941, 1943, 1945; CALLAN, 1942). It may also prevent the chromosome division. In this way, bridges and fragments may be produced in the mitotic anaphase owing to the fact that the centromeres are struggling in opposite directions, whereas the distal parts of the chromosomes, which an earlier cold chock has prevented from dividing, still hang together (DARLINGTON and LA COUR, 1940). Corresponding pictures have been observed also by the author (Plate III, Fig. 2; Fig. 45). As regards the bearing of these phenomena on the time for the division of the chromosomes, see p. 316.

e. Heterochromatin.

New light has been thrown on the problems connected with the heterochromatin by the recent experiments with nucleic acid starvation that have been made especially by the JOHN INNES school (DARLINGTON and LA COUR, 1940; CALLAN, 1942). A detailed analysis of the heterochromatic segments in various *Triturus* species has been made by CALLAN (1942). According to him, the heterochromatin in these forms is located chiefly round the centromeres. I have obtained similar results by cold treatment of *Triturus vulgaris* and *cristatus* according to CALLAN's method. I therefore confine myself to mentioning that both species have centric heterochromatic segments, and that the entire proximal third part of the longest arm in the largest chromosome of *Triturus cristatus* is heterochromatic. In endeavouring to discover the factors involved in the localization of the heterochromatin, these observations are of some interest (*v. infra*).

Similar results can be obtained by starvation. Males of *Triturus vulgaris*, having been kept without food from the middle of May to the middle of July — which they could stand without difficulty —, showed distinct heterochromatic segments in consequence of nucleic acid starvation (Fig. 42).

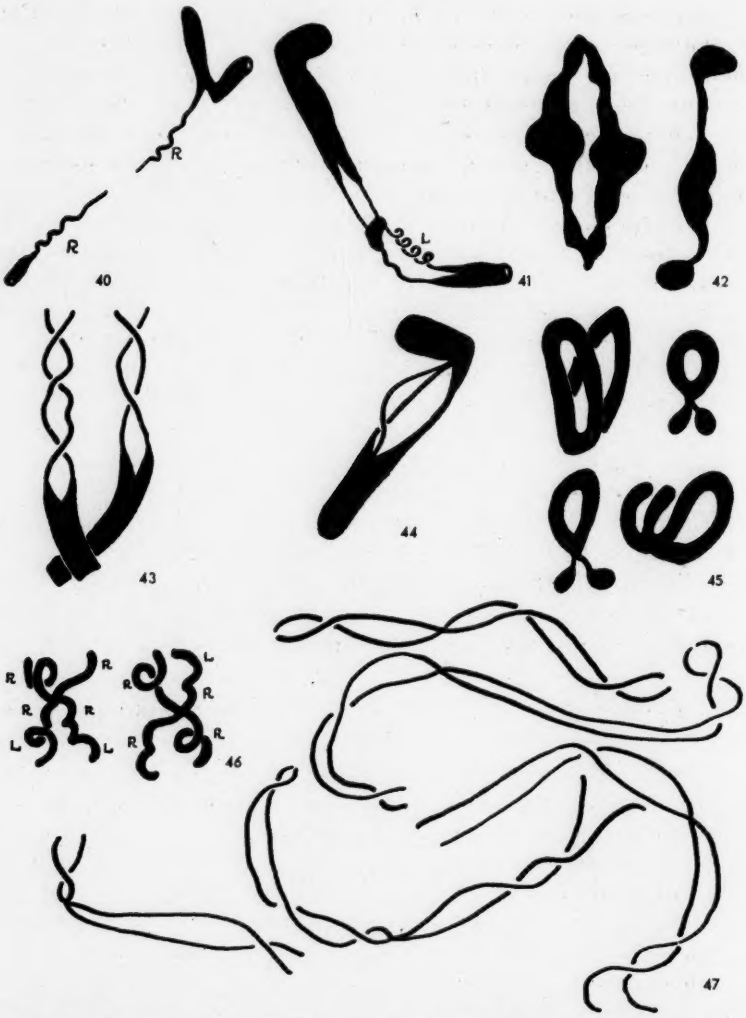


Fig. 40. The same as Plate III, Fig. 7. Camera lucida drawing. (5000 ×). — Fig. 41. Mitotic spiral of *Protopterus annectens*. — Fig. 42. Meiotic chromosomes of *Triturus vulgaris* showing nucleic acid starvation. — Fig. 43. The same as Plate III, Fig. 8. Camera lucida drawing. (5000 ×). — Fig. 44. The same as Plate III, Fig. 9. Camera lucida drawing. (5000 ×). — Fig. 45. Mitotic chromosomes of *Triturus vulgaris* after cold treatment. The chromatids hang together at the distal ends. — Fig. 46. Two M^{II} chromosomes of *Rana arvalis* treated with 0,1 % KOH, 5 minutes. Despiralization of the major spiral. N. B. the changes in

Corresponding conditions in this respect are found also in Anura. In the latter forms, however, an analysis of the heterochromatic segments is greatly complicated by the double spiral during meiosis, which conceals them at diakinesis and metaphase. Nevertheless, in *Bufo calamita* and *viridis* as well as in *Rana esculenta* I have managed to obtain a few rather good mitosis pictures, which show that in this group, too, the heterochromatic segments are proximally located (Fig. 14).

This localization of the heterochromatic segments may be explained as resulting from the position of the chiasmata in these forms. Experience has shown that those parts of the chromosomes which are seldom or never subjected to crossing-over are often heterochromatic (MULLER, 1916; DARLINGTON, 1937, p. 330; CALLAN, 1942). True that, as CALLAN also observes, chiasmata are sometimes formed in the heterochromatic segments of *Triturus cristatus*, but this is a very rare occurrence. The origin of the more distal heterochromatic segments which not infrequently occur in the *Triturus* species (CALLAN, *op. cit.*) cannot be explained in this way. I am inclined to believe that they have been formed in connection with secondary chromosome modifications as segmental interchanges, etc., whereby originally centric heterochromatic segments have in part been shifted further distally. Analyses of several species and especially local races and hybrids of *Triturus* may perhaps serve to throw light on this question.

B. COLCHICINE TESTS AND THE QUESTION OF POLYPLOIDY IN URODELA AND ANURA.

Some preliminary tests with colchicine have been made on *Triturus cristatus*. They have so far been merely intended to determine the suitable dosage, etc. for future larger series. However, some interesting facts, which seem to deserve mention, have already been elucidated by these experiments.

The newts, which were in the most active period of spermatogenesis (middle of July), were twice injected subcutaneously with 1 cc 0,50 % colchicine solution, with an interval of one day. On the day after the last injection they were decapitated and the testes were prepared in accordance with the method adopted in this study. In the preparations, only meiotic stages were investigated. *At zygotene and pachytene* the

direction of the spiral. — Fig. 47. 5 chromosomes from mitotic metaphase of *Ambystoma mexicana* treated with 0,1 % KOH, 5 minutes. Strong relational coiling between chromatids. The position of the chromatid ends indicates that end rotation causes relational coiling in this case.

effects of the drug are already quite perceptible, in that *the polarization*, which otherwise is characteristic of these stages (p. 262), *has completely disappeared*. Since the visible number of chromosome units is the haploid number, the chromosomes at these stages being paired, I have interpreted this as *an effect of the colchicine on the centrosome*. This matter is discussed in the chapter on »the bouquet». At later stages the effects of the colchicine are the usual ones (cf. LEVAN, 1938, 1939). The spindle is not developed, the bivalents lie scattered in the nucleus at a considerable distance from one another, much as in diakinesis. The chiasmata are terminalized in increasing degree, whilst the spiralization gradually increases and becomes much more marked than normally. This must probably be viewed in connection with the fact that the cell division is stopped by the disturbance of the spindle mechanism, so that the spiralization of the prophase is not interrupted. — According to ÖSTERGREN (1944), however, colchicine contracts the protein fibre of the chromosome, *i. e.* the contraction observed should be a direct function of the drug. — The degree of terminalization of the chiasmata is also increased (Plate III, Figs. 3 and 4). As regards spiralization, the conditions resemble those obtained in mitosis by colchicine treatment (BARBER and CALLAN, 1942, according to DARLINGTON and LA COUR, 1942).

Similar results have been obtained by treatment with acenaphthene (SWANSON, 1941) and by freezing (BARBER and CALLAN, *op. cit.*; BÖÖK, 1945). In regard to terminalization of the chiasmata and meiotic spiralization, the results with colchicine resemble those entailed by the action of low temperature.

The chromosomes gradually separate from one another without cell division, and diploid restitution nuclei are formed (Plate III, Fig. 3), which afterwards pass through the second division. The latter may also be affected in a corresponding manner. Thus, owing to the effect of the colchicine, spermatozoa with 2n or 4n chromosomes — depending on whether only one division or both of them have been affected by the drug —, are produced. Thus colchicine treatment is undoubtedly an effective method for producing polyploid spermatozoa in *Triturus*. Whether these spermatozoa can generate polyploid offspring is another question. In that case such offspring (if the egg is haploid) would be triploid or pentaploid.

As to this question, two different possibilities must be taken into consideration. Firstly, these polyploid spermatozoa may be incapable of fertilization; secondly, it is conceivable that any polyploid embryos

which may have been formed are not viable. In regard to the first of these contingencies, we still know nothing; as regards the second, on the other hand, we have some basis for a judgment. A number of triploid Amphibians, found in nature or experimentally produced, are actually known. Higher polyploids have also been occasionally observed (Table 16).

From the above it is evident that there is nothing to prevent a triploid, tetraploid or even a pentaploid or hexaploid from being viable. It has long been recognized that polyploidy in animals is a rarity and mainly confined to parthenogenetic or hermaphrodite forms (cf. VANDEL, 1937; SUOMALAINEN, 1940). This has been explained in accordance with MULLER's (1925) theory, based mainly on the conditions in *Drosophila*, that the sex chromosomes in bisexual animals cause intersexuality in the polyploids. This theory has met with rather wide acceptance. The criticism levelled against it by certain authors, such as WESTERGAARD (1940) and LÖVE (1944), however, points out that polyploidy need not cause intersexuality if the sex determining genes are situated as in *Melandrium*. Even if Anura and Urodela had a sex-determining mechanism of the *Melandrium* type there are, however, reasons speaking against polyploidy as a factor in the evolution of species and races in the Amphibians:

(1) Though, as already indicated, triploids may occasionally occur, they can scarcely give rise to balanced polyploid offspring.

(2) Allotetraploids occur extremely seldom in animals (SUOMALAINEN, 1940); at any rate they have not been found in Anura or Urodela. The explanation seems to lie partly in the pairing instincts of most animals, which render crossings very rare, partly in the conditions of growth, etc. in animals as compared with plants; the latter having greater opportunities for somatic doubling with resulting tetraploid shoots, which can then develop diploid gametes (cf. HUXLEY, 1942, pp. 43, 45, 87, 140 ff.). Moreover, artificial insemination leads to hybrid offspring only in extremely rare cases (G. HERTWIG, 1918; TCHOU SU, 1931; P. HERTWIG, 1936; DÜRKEN, 1938); the reason being that the sperm nucleus in the foreign egg plasma degenerates. Instead, the thus activated egg nucleus is often diploidized, whence such crossing results in normal diploids, resembling the mother species in every respect. G. and P. HERTWIG's (1920) experiments with *Rana esculenta* show that the duplication of chromosomes involved in the diploidization is due to a special capacity for adaptation in the haploid. Diploid eggs, after fertilization with sperm foreign to the species, apparently do not

TABLE 16. *Polyploidy in Anura and Urodela.*

Species	Degree of polyploidy	Origin	Author
Rana esculenta ...	3n	Egg from a ♀, which after fertilization with sperm from another species produced 2n-larvae; after fertilization with *R. e.* sperm 3n-larvae, both types resembling the mother. Probably non-reduction in ♀.	G. and P. HERTWIG (1920)
» » ...	3n	A larva with the chromosome number 39. Untreated. Probably non-reduction in ♀. Another larva with the chromosome number 36. Probably the same origin as the preceding specimen, but with the chromosome number modified »... de la suppression traumatique de quelque chromosomes dans une figure de maturation du type diploide ...» (DALCQ, 1930).	DALCQ (1930)
» »	3n	Four adult males from a natural population in the island of Bornholm. In the same population diploids were also found. Probably originated in the same way as the preceding specimens, or possibly by cold shock.	WICKBOM (this paper)
» *nigromaculata*	3n	Parthenogenetic tadpoles.	KAWAMURA (1939 a, b)
» »	4n	» »	»
» »	6n	» »	»
» *pipiens*	3n	» »	PARMENTER (1933)
Eurycea bislineata	3n	Larvae from natural populations.	FANKHAUSER (1939 a, b)
» »	4n	Larvae from natural populations.	»
Triturus palmatus	3n	Larvae. Origin unknown.	FANKHAUSER (1934)
» *viridescens*	3n	» » »	FANKHAUSER and KAYLOR (1935)

Species	Degree of polyploidy	Origin	Author
Triturus viridescens	3n	Larvae from natural populations and after cold shock.	FANKHAUSER (1938 a, b, 1939 a, b); GRIFFITHS (1941)
Triturus viridescens	5n	One larva from natural population.	FANKHAUSER (1941 a, b)
Triturus vulgaris	3n	Adult male from natural population.	BÖÖK (1940, 1945)
» »	3n	Larvae developed after cold shock.	BÖÖK (1943, 1945)

double their chromosome number, but continue their development with the normal diploid number of chromosomes.

In Urodela the possibilities of crossings of species are evidently somewhat larger [see LANTZ (1934) for literature] but by no means as large as among some plants. Without hybridization, allopolyploidy is impossible; thus one more avenue open to the vegetable kingdom for the development of species and races by polyploidy is practically closed to Anura and Urodela.

(3) Another circumstance which further restricts the capacity of polyploids for playing any part in the development of species in these forms is that polyploids, unlike plants, do not show any greater size and vitality than the corresponding diploids, whence they have no greater capacity for asserting themselves in competition with the latter: rather the contrary (FANKHAUSER, 1941 a, b). This is doubtless connected with the essentially different process of growth in most animals, on the one hand, and plants, on the other. A polyploid animal has therefore no advantage over a diploid. — It is a curious fact that the polyploid *cells* in Urodela are considerably larger than the diploid (FANKHAUSER, 1941 b; BÖÖK, 1940, 1945), although the organism as a whole is not larger. I have made a similar observation in *Rana esculenta*.

It is thus evident that, despite the rather frequent occurrence of spontaneously developed polyploids [about 1 per cent. in *Triturus viridescens*, 10 per cent. in *Eurycea*, according to FANKHAUSER and GRIFFITHS (1939)] and the ease with which polyploids can be experimentally produced in Amphibians, polyploidy can scarcely play any part in the development of species and races within Anurans and Urodelians. The study of natural or experimentally produced polyploids can nevertheless give us a good insight into a number of hitherto

obscure questions. In particular, it should be possible in this way to cast new light on certain problems relating to the determination of sex (cf. Ch. III). The colchicine tests on Urodela will accordingly be continued with that object in view.

C. CHROMOSOME STRUCTURE.

a. Number of threads, etc.

Though this investigation was not originally intended to include a study of the finer chromosome morphology and chromosome structure, the author, in the course of the work, has been confronted with problems, to the solution of which he believes himself able to contribute.

An animated discussion has long been carried on in regard to the exact phase in which the longitudinal division of the mitotic chromosome into chromatids occurs and in regard to the number of threads (»chromonemata») of the chromosomes at various stages. For reasons of space, I cannot enter into any detailed survey of the literature on the subject. Good summaries of it will, however, be found in DARLINGTON (1937), GEITLER (1938), KUWADA (1939), and NEBEL (1939).

The different views may be briefly summed up as follows:

(1) The metaphase chromosome at mitosis consists of two single chromatids. The anaphase chromosome at mitosis consists of one single thread. The division takes place during the resting stage (DARLINGTON, 1937).

(2) The metaphase chromosome at mitosis consists of two bipartite chromatids. The anaphase chromosome at mitosis consists of two threads. The division takes place at metaphase (HUSKINS, 1937), or during the resting stage (GEITLER, 1938; CREIGHTON, 1938).

(3) The metaphase chromosome at mitosis consists of two quadripartite chromatids. The anaphase chromosome at mitosis consists of four threads. The division takes place during the resting stage (NEBEL, 1939; KUWADA, 1939).

The advocates of these various theories base their views mainly on microscopical investigations.

Experiments with x-ray radiation have also been made and dragged into this discussion; their results, however, are not sufficiently convincing. DARLINGTON (1937) attaches great importance to MATHER and STONE's (1933) and RILEY's (1936) x-ray experiments; but later investigations by NEBEL (1939), though they do not actually confute DARLINGTON's opinion, can scarcely be interpreted as supporting it. GEITLER (1940) denies the value of x-ray experiments in this connection,

but recent investigations by NEWCOMBE (1942) greatly support DARLINGTON's views.

Nor has the electron microscope hitherto revealed anything new in regard to these problems (ELVERS, 1943).

DARLINGTON (1937), in support of his view, points out that the structures in question are so fine that they lie at, or close to, the limit of the resolving power of the microscope. Experiments made by HRUBY (1934) with infra-red light show that, if the size of the structure under examination stands in a certain relation to the wave length of the light used, pictures which appear to reflect doubleness in the structure are produced, although such doubleness does not actually exist.

However, in recent years Japanese and other investigators (*e. g.* KUWADA, 1938; AISIMA, 1940, COLEMAN, 1940) have published pictures, obtained by pre-treatment of the material with media of higher pH than that usual in the division stages, with the object of uncoiling the chromosome spirals and thus obtaining facilities for studying their morphology. In these pictures, doubleness in the anaphase chromosomes and cloven chromatids in the mitotic metaphase is distinctly observed. AISIMA (*op. cit.*) definitely states that such structures are not seen in untreated material.

I have made similar observations as regards the large mitotic chromosomes in *Ambystoma*, *Triturus*, and *Protopterus*. *In untreated material there is not the slightest indication of doubleness in the structure of prophase and metaphase chromatids*, even where the spirals have been straightened out by mechanical means (Figs. 40 and 41; Plate III, Fig. 7). This is in accordance with WADA's (1933) results by mechanical stretching of the chromosome spirals. *In material pretreated with KOH, $NaHCO_3$ or NH_4OH, however, these duplications are brought out clearly* (Figs. 43 and 44; Plate III, Figs. 8 and 9). As shown by the pictures, the two threads here lie so far apart from one another (ca. 1,5 microns) that the doubleness cannot have been produced in the manner suggested by HRUBY (1934).

There are two possible explanations of the differences between the treated and untreated material. Either these double chromatids represent the natural condition, which the alkali treatment brings out distinctly enough to enable it to be observed; or else this doubleness is produced by the alkali treatment itself.

Now, what is the evidence in support of the one or the other of these two alternatives? Certain investigators who have *not* resorted to alkali treatment (*e. g.* DEARING, 1934; JEFFREY, 1937, etc.) maintain

that they have observed the said doubleness in the mitotic chromosomes. Their pictures, however, are not sufficiently clear to carry conviction. Indeed, DARLINGTON's view that the supposed »doubleness» must be attributed to the inadequate resolving power of the lenses employed to seems to be applicable in such cases. I have never seen microphotographs which clearly show such a doubleness without a preceding alkali treatment. Other *investigators*, too, *who* have dealt with these problems and *have been in a position to compare preparations* of the same nature *with and without alkali treatment definitely state that the doubleness is observable only in material thus treated* (AISIMA, 1940).

However, in my cold-treated material I have observed pictures which may contribute to the settlement of these questions. As previously shown (DARLINGTON and LA COUR, 1940; CALLAN, 1942, WICKBOM, present paper, p. 307), cold treatment tends to prevent the division of the chromosomes. Bridges and fragments are frequently observed in mitoses after such treatment (Plate III, Fig. 2). Moreover, it may conduce to inactivate the spindle (*v.* Ch. II A d), so as to impede the mitosis itself. Thus I have observed that in material from *Rana esculenta* fixed two hours after treatment for 48 hours at 4° centigrade, there are no stages later than metaphase. In material fixed immediately after such cold treatment there are no mitotic stages at all. It is thus evident that the mitosis is prevented by cold.

In the prophase and metaphase of material fixed two hours after cold treatment, I have observed that the chromatids still cohere, especially at the distal parts (Plate III, Fig. 2; Fig. 45). *In my view, the only possible explanation is that these chromosomes, owing to the cold treatment, have been prevented from dividing completely. This division must have occurred during the treatment, that is, in the preceding resting stage.* — It is scarcely within the bounds of possibility that the nuclei during the two hours after the treatment should have completed their mitosis and started a new one, especially as in this material no later stages than metaphase had been observed. The same remark applies to material taken one hour after the treatment, although not even metaphases occur there.

Thus, I consider it proved that the chromatids observed at prophase and metaphase have been separated from one another by the longitudinal division of their mother chromosome in the immediately preceding resting stage. This is in complete accordance with DARLINGTON's (1937) view.

The doubleness which the author and others have observed in alkali-treated material must thus have resulted from the said treatment. Now, it has long been known that alkali conduce to uncoil the chromosome spirals, and it is precisely owing to such effect of high pH that this treatment is adopted. In regard to spiralization, the chromosomes are thus, so to speak, carried back through the prophase to a resting stage. KUWADA and NAKAMURA (1934) as also WADA (1937) have in fact succeeded in artificially producing a complete resting stage by alkaline treatment. The mechanism of this phenomenon is, generally speaking, unknown, but it is significant that YAMAHA's (1935, 1938) measurements of the pH in the nucleus show that it is highest during the resting stage and lowest at metaphase and anaphase. I therefore consider that alkali treatment, owing to its high pH, brings about changes in the cell and chromosomes which are normal in the resting stage, *whence the same phenomena occur as during a normal resting stage, namely uncoiling of the spirals and the division of the chromosome into chromatids.*

b. The bouquet.

The bouquet stage, as we know, signifies that during the meiotic prophase, especially at zygotene and pachytene, the chromosomes are polarized so that one or both ends of all of them are concentrated towards a point at the periphery of the nucleus, whilst the remaining parts of the chromosomes are freely distributed in the nucleus in the form of long loops. It is also significant that, as pointed out by DARLINGTON (1937, p. 90), the ends are polarized whether the centromeres are situated there or not. DARLINGTON does not attempt to explain this polarization. He merely supposes (*op. cit.*, p. 91) that it should be regarded as an adaptation in order to secure regular pairing.

Certain attempts to explain this polarization have, however, been made by other authors. According to RABL (1885), it is a relic after the chromosome arrangement in the preceding telophase. It seems to me, however, that his explanation which has been supported by ATWOOD (1937), WU (1938), and HIRAOKA (1941) does not completely cover the said phenomenon. Firstly, in my opinion, it does not explain why all ends have polarized whether the centromere is situated there or not (cf. DARLINGTON, 1937, p. 91). Secondly, this theory does not explain why the polarization is abolished in colchicine-treated material (*v.* Ch. II B). This might be due to inhibition of the preceding division. However, the size of the nuclei as compared with the normal as well as

an approximative count of the number of chromosomes in these nuclei show that the number of elements is the haploid number, whence the preceding division must have been normal. This explanation is thus unsatisfactory.

On the other hand, the polarization has been explained as an effect of the centrosome. In the cases examined (GELEI, 1921; DARLINGTON, 1936), the centrosome seems in fact to be situated at the base of the bouquet. The objection to this explanation is that centrosomes have not been discovered in higher plants where polarization has been found (KIHARA, 1927; BĚLAŘ, 1929; HIRAOKA, 1941). However, we do not yet know whether the absence of centrosomes in the said material is real or merely apparent, possibly owing to their minuteness. This theory regarding the dependence of polarization of the centrosome, which has been espoused especially by GELEI (1921), is strongly borne out by my own observations in colchicine-treated *Triturus* material. There, as previously stated, the polarization has been abolished in all the phases of meiosis. As we know, colchicine inactivates the spindle and as the centrosome is closely connected with the development of the spindle in the more primitive plants and all animals, it seems reasonable to suppose that the non-polarization in colchicine-treated material is connected with a colchicine effect on the centrosome. How the action of the centrosome on the chromosome ends normally proceeds, and how this effect is neutralized by the colchicine, I am unable at present to explain. Generally speaking, we are in the dark regarding the forces which operate during cell division, and theoretical speculation, which must obviously be based on very loose grounds, will not conduce to extend our knowledge.

c. Spiralization.

In regard to the spiralization of the chromosomes, certain preliminary observations, which deserve mention, have been made. In Chapter II C a it has been shown that the division of the chromosomes into chromatids normally occurs in the resting stage, but under the action of KOH can be produced in other stages. The pictures obtained by such treatment (Fig. 43; Plate III, Fig. 8) show that the plane of division was screw-shaped, so that the daughter chromatids from the moment of »birth» are relational coiled. This is in conformity with DARLINGTON's (1937) view regarding the origin of relational coiling.

Another type of relational coiling has also been found in material treated with KOH. In metaphases thus treated, the chromosomes often

still lie in a typical metaphase orientation. However, they are longer and thinner than normally, and the spiralization seems less compact. Moreover, the chromatids are markedly relational coiled. This relational coiling is densest at the ends of the chromosomes and must have been produced by the rotation of the chromosome ends round one another, during the uncoiling of the minor spirals. The occurrence of such rotation is in fact distinctly indicated by the position of the chromatid ends in some of the pictures (Fig. 47; Plate I, Fig. 4). These observations conduce to bear out DARLINGTON's (1937) theories regarding torsion as spiralizing factor in the chromosomes. However, this despiralization with KOH is so far abnormal in that the minor spiral has not been uncoiled, as in the normal telophase-resting stage, by increase of amplitude and transition to a relic spiral (DARLINGTON, 1937), but by end rotation and transition to a relational spiral. This may be due to the differences between the single chromosome threads at telophase and the two cohering chromatids (each of which may possibly itself be divided), after KOH treatment, at metaphase.

In untreated material the minor spiral at mitotic metaphase has also been observed. Earlier observations of this nature are extremely rare, as the spiral is usually so dense that the chromatid looks like a compact cylinder. In attempts to unloosen this compact coil with alkali, other, above indicated, changes in the chromatid, which reduce the value of such observations of the spiralization, often occur. In untreated material the minor spiral during mitosis has been observed by GEITLER (1935) in *Allium* and *Gasteria* and by UPCOTT (1936) in *Tulipa*. Mention should also be made of WADA's (1933) admirable micrurgic study of *Tradescantia*, where the chromosome was drawn out with micro-dissecting needles, so that the spiral could be made accessible for observation.

My own observations have been on similar lines. In squash preparations it occasionally happens that a chromosome which is exposed to pressure during the preparation cannot float away in one direction or another, as one of its ends or both have chanced to get anchored. In Fig. 40 and Plate III, Fig. 7 we see that one end of an *Ambystoma* chromosome at mitotic metaphase has got caught in a grain of yolk, so that the centromere and three chromatid arms are fastened there, whilst the fourth, which has stuck also at the other end, has been drawn out into a long thread, in which a number of right hand spiral gyri can easily be observed under the microscope. In the microscopic picture the thread is undoubtedly single.

A similar case is shown in Fig. 41 (*Protopterus*). Here the centric parts of the two chromatids are drawn out, so that their right hand minor spirals can be clearly observed. The undivided centromere is also distinctly visible as a bridge between the two chromatids. Here, too, the chromatids seem to be single under the microscope.

As previously pointed out (Ch. I B, on meiosis), it could be shown that the major spiral is lacking in Dipnoi and Urodela. On the other hand, it occurs in Anura and *Emys*. During the second meiotic division it could be despiralized with KOH and was found to be formed of chromatids of the usual mitotic thickness (Fig. 46). Moreover, as will be observed, changes in the direction of spiralization occur in the major spiral (cf. MATSUURA, 1941; SPARROW and WILSON, 1941).

These phenomena will be subjected to further investigation, in the hope that they may contribute to the solution of the problems relating to the mechanism of chromosome spiralization. At the present stage, therefore, I will not enter into a discussion of these preliminary observations. Good summaries of the literature on this subject will be found in KUWADA 1939 and MATSUURA (1941).

III. THE SEX CHROMOSOMES IN DIPNOI, URODELA, ANURA, AND EMYS.

Setting aside some antiquated literature (see OGUMA and MAKINO, 1937), where it is asserted that in the Amphibians the males have XO, there are now two rather widespread views regarding the sex-chromosome conditions in that group of vertebrates. As regards the sex chromosomes in the Dipnoi, all that is hitherto known is that AGAR (1911, 1912) has not found anything which indicates the occurrence of heterochromosomes in the male of *Lepidosiren paradoxa*.

According to the earlier of these two views, which is represented by WITSCHI (1922, 1923, 1924, 1929, 1933), IRIKI (1928, 1929, 1930, 1932 a, b), MINOUCHI and IRIKI (1931), MAKINO (1932 a, b), SATO (1934, 1936 b, 1939), and BUSHNELL *et al.* (1939), the male in Anura, during the first division of meiosis, has a chromosome pair which, in view of its appearance and behaviour, has induced the said investigators to regard it as a pair of sex chromosomes. According to WITSCHI, it is an XY pair, whereas IRIKI believes it to be an XX pair. Whilst MAKINO considers himself unable to decide which of the two alternatives is correct, SATO endorses IRIKI's opinion.

The other view is represented principally by GALGANO (1933), who states that the males of the species of Anura and Urodela examined by

him have no sex chromosomes detectable under the microscope. STOHLER (1928) and SAEZ *et al.* (1936) report the same observation in four Bufonids. This view has been sharply criticized by WITSCHI (1933), who maintains that heterochromosomes are morphologically distinguishable during the meiosis in *Bufo vulgaris* and other *Bufo* species. He even considers that these heterochromosomes can be homologized with the sex chromosomes he believes himself to have found in *Rana temporaria.* SATO also states (1936 b, p. 960) in regard to the sex chromosome of *Bufo radderi*: »And also this chromosome is evidently homologous with that found in several Japanese and American toads».

There are, moreover, some statements (KING, 1912; PARMENTER, 1919; CARRICK, 1934) to the effect that the sex chromosome or chromosomes in Urodela are attached to an autosome. This view has been criticized by IRIKI (1932 c), SATO (1939) and others, and must be considered to have no foundation. Otherwise all the investigators who have studied the chromosomes in Urodela are agreed that in the males of the species examined, there are no morphologically or behaviourly recognizable sex chromosomes (see OGUMA and MAKINO, 1937, for literature).

Now, what are the observations which form the basis of the two conflicting views regarding the existence of sex chromosomes in Anura? Those authors who maintain the occurrence of sex chromosomes in the male during meiosis have observed a chromosome pair which in the first meiotic division is unlike the others in form and behaviour. It is described by IRIKI (1932 a, p. 67) as follows: ». . . a large V-chromosome lying always vertically in the periphery of the equatorial plate and taking the form of a vertical V, while the other large chromosomes show always a form of vertical or tangential ring. This vertical V-chromosome is composed of two atelomitic homologous diads». Similar observations, broadly speaking, have been made by all the above-mentioned authors who support IRIKI's or WITSCHI's view. WITSCHI also maintains (1922) that the members of this pair separate before the other chromosomes.

According to GALGANO (1933), the appearance of this chromosome pair cannot have anything to do with its supposed sex-chromosome character, seeing that in certain cells two, three or more such pairs occur. Furthermore he shows that, when only one such pair is found, it is not always the same pair in different nuclei of the same species or the same individual. These views are endorsed by SAEZ *et al.* (1936).

In his latest study of this question SATO cites the said investigators but adds (1939, p. 65): »But the existense of »the odd» chromosome among anurans seems to suggest something of the phylogenetical evolution of the sex-chromosomes in the amphibians». He thinks it probable that recognizable sex chromosomes are lacking in Discoglossidae, but that they exist in the higher Anurans.

Also in regard to the existence of sex chromosomes in Reptilia, there has previously been a great divergence of opinion. Now, however, thanks to the many excellent studies published by MATTHEY, NAKAMURA, and OGUMA (see OGUMA and MAKINO, 1937), it may be regarded as established that in the males of examined species there is no chromosome pair which gives us reason to suspect the occurrence of heterochromosomes during meiosis or mitosis. OGUMA (1937) has also shown that female heterogamety of the XO type, a feature which has long been known to exist in birds, occurs also in reptiles.

Own results. — In the male of *Emys* I have not found any heterochromosomes, whence I can merely confirm the views above cited. As regards Anura, Urodela, and Dipnoi, I have in several cases, especially in the Anurans, observed »the odd chromosome», but I share GALGANO's opinion that its form and behaviour has nothing to do with its supposed sex-chromosome character. Here we are simply concerned with a chromosome pair with submedian or subterminal centromeres, whose shorter arms do not always develop chiasmata, and which during the first division of meiosis, usually is held together only at the longer arm. This accords with GALGANO's view (1941, p. 161): ». . . *una commune tetrade con un solo chiasma terminale*». GALGANO, however, does not explain why one of the arms fails to develop chiasmata, whence his evidence is not complete. The absence of pairing in one arm might, of course, be regarded as a distinct indication of an XY pair. But, as shown by my curves and tables for the relation between chiasma frequency and chromosome or arm length (*v.* Ch. I B), none of the chromosomes in the males of any species have an arm which deviates more markedly from the others than may be due to mere chance — except possibly in *Triturus cristatus* and *Hyla arborea*, where, however, the low pairing frequency can easily be explained in another way (pp. 285—286). That it cannot be due to heteromorphism is indicated by the occasional formation of more than one chiasma in these arms (see Table 5).

Thus no cases of failure of pairing owing to heteromorphism between the members of a chromosome pair have been found in any

species of Anura. As previously shown, *the fact that arms are sometimes unpaired is due to a restricted time limit, with a consequent low chiasma frequency in these forms.* No heteropycnosis which might be attributed to sex chromosomes has ever been observed in the meiotic prophase. Nor have cold experiments shown the occurrence of heteromorphism in any pair as regards heterochromatic parts. CALLAN (1942), indeed, states that he had found in one plate of *Triturus* a chromosome pair which was heteromorphous in regard to a constriction: this observation, however, is obviously not of general validity.

A large number of embryos of *Triturus cristatus*, *T. vulgaris*, *Bufo vulgaris*, *Discoglossus pictus*, *Rana arvalis*, and *R. temporaria* have been examined by the present writer, and much labour has been bestowed on ascertaining whether any heteromorphous chromosome pair occurred in any of these embryos. Not in a single case could such a pair be discovered. In the ovaries of *Triturus cristatus*, *T. vulgaris*, *Bufo vulgaris*, *Rana arvalis*, and *R. temporaria* a number of oögonium divisions were also examined. In all of them I found regular chromosome pairs, without any indication of heteromorphism.

Accordingly I consider it proved that *in Anura and Urodela nothing has been brought to light which even suggests the occurrence of morphologically or behaviourly recognizable heterochromosomes in either sex.* WITSCHI's (1933) attempt to homologize the »sex chromosomes» of the Ranids and Bufonids thus, of course, falls to the ground. The same applies to SATO's (1936 a) view regarding the homology of these chromosomes in different Bufonids. *In Dipnoi* I have thoroughly analysed the meiosis and mitosis of the male only, and I must therefore confine myself to stating that *recognizable sex chromosomes do not exist in the male.*

Anura and Urodela deviate in this respect from Amniota and correspond, instead, to Teleostei, where the same conditions have been found (MAKINO, 1939; WICKBOM, 1941, 1943). *Thus, in all forms of Anamnia hitherto examined the sex chromosomes, if they exist at all, seem to be so undeveloped that they cannot be distinguished, in shape or behaviour, from the autosomes.*

WITSCHI (1923, 1929) found that in *Rana temporaria*, after the fertilization of eggs from a hermaphrodite with sperm from a male of Davos race, males and females were produced, whereas after the fertilization of eggs from a female of Davos race with sperm from the hermaphrodite only females were generated; he therefore considers that *Rana temporaria* has a heterochromosome mechanism of the

Drosophila type. CREW (1921) likewise considered, for genetical reasons, that the male of *Rana* was heterogametic. This, however, seems to conflict with the fact that parthenogenetic specimens of *Rana* may be of either sex, although the females appear to be in the majority (LOEB, 1918; GOLDSCHMIDT, 1920; PARMENTER, 1920, 1925; KAWAMURA, 1939 a, b).

The genetic facts just mentioned and the polyploidy conditions in Anura (p. 311) by no means conflict with my conclusion, which has been arrived at on cytological grounds. On the contrary, the facts that parthenogenetic Ranids may be of either sex and that triploidy does not adversely affect the males of *Rana esculenta*, although genetic experiments point to the male heterogamety, conduce to bear out my view. Such conditions would be very difficult to explain if these forms had sex chromosomes so well developed that they could be distinguished under the microscope by their morphology or behaviour.

As regards Urodela the conditions are rather different. Many triploids are known (Table 16) and they are of both sexes although the male is quite normal (BÖÖK, 1940, 1945), whereas the ovaries of the females are small and undeveloped (FANKHAUSER, 1938 b, 1940): for the above indicated reasons, this would suggest female heterogamety. Many experimentally produced haploids are known (see GRIFFITHS, 1941, for literature), but as a rule they have not been sufficiently developed to enable us to obtain a reliable sex quota. Thus, if the conditions in the triploids of Urodela may be interpreted on the analogy of those in *Drosophila*, this would be a clear indication of female heterogamety. My cytological results, however, show that in this case the morphological difference between the members of this heterochromosome pair must be very small indeed, since it could not be detected with the methods adopted here. It is at any rate absolutely certain that the same conditions as in some Sauropsida with XO in the female do not exist in Urodela.

Anura and Urodela may thus be said to correspond as regards the fact that heterochromosomes in either sex are not recognizable under the microscope, but differ in so far as genetic and other observations point to female heterogamety in Urodela, whereas in Anura nothing definite can be stated as to which sex is heterogametic.

IV. PHYLOGENY.

The cytological conditions in the Anura, Urodela, and Dipnoi examined, seem to me to point to a certain phylogenetic relationship

TABLE 17. *Occurrence within different groups of certain cytological features.*

Feature	Dipnoi	Urodela	Anura	Chelonia	Other Reptilia
Chromosome length at mitotic metaphase in microns	5—30	7—30	3—19	0,5—7	0,5—10
n =	17—19 [1]	12—32 [1]	11—19 [1]	25—32	11—25
Type of spindle	Hollow	Hollow	Dense	Dense	Dense
Meiotic spirals	Minor	Minor	Minor and major	Minor and major	?
Terminalization coefficient in M^I	0,58	$<0,65$	$>0,88$	$>0,88$	?
Correlation between chiasma frequency and length of chromosomes	Positive	Positive	—[2]	—	?
Initiation of pairing	Preterminal	Preterminal	Preterminal	Preterminal	?
Localization of heterochromatic segments	?	Proximal	Proximal	?	?
Sex chromosomes	?	Cytologically not discernible		XO—XX	XO—XX
Set differentiated into macro- and micro-chromosomes	—	—	+[2]	+	+++

between those groups. Cytology as an auxiliary to embryology and comparative anatomy, when it comes to the unravelling of such remote relationships, is, however, a rather untried science. Judgments based thereon should therefore be passed with the greatest caution. In the majority of cases we should content ourselves with examining how far cytology can confirm phylogenetic findings substantiated or rendered probable in other and better explored ways. Where some reasons weigh in favour of a certain view and others against it, we may perhaps venture to lay the cytological findings in the balance, though this, too, must be done with great discrimination.

Cytological peculiarities common to the said groups of vertebrates are the following (cf. Table 17):

(1) General chromosome morphology. The chromosome are large and, with few exceptions (Cryptobranchoidea, *v.* Ch. I A), distinctly two-armed.

[1] Cf. p. 258. [2] + = evident, +++ = very evident, — = feature missing in that group.

(2) Chromosome number. It varies considerably between these groups and even within them, but, as shown in Chapter I A, the different chromosome numbers in Urodela and Anura may be ordered into evolutionary lines.

(3) Pre-terminal initiation of pairing and rather restricted time limit (Ch. I B), and in consequence of this, distal localization of the chiasmata in all species except *Protopterus*, where the time limit seems to be somewhat wider. This is a primitive feature, from which the conditions in the other species may have been derived (cf. p. 285 and DARLINGTON, 1937, p. 295). These forms are also marked by an unusually low chiasma frequency relatively to the chromosome length and by proximal heterochromatic segments; features which are undoubtedly connected with the facts just mentioned.

(4) Very similar reaction in chromosomes, spindle, etc., to temperature effects (Ch. II A).

(5) Lack of cytologically recognizable sex chromosomes (Ch. III). This, however, is a feature which these forms have in common with other Anamnia investigated (WICKBOM, 1941, 1943), though they diverge in this respect from Amniota (see OGUMA and MAKINO, 1937, for literature).

Even if we are thus justified in maintaining that the cytology of these forms strongly bears out the view as to their phylogenetic connection, there are also cytological findings suggestive of their grouping into smaller divisions. With respect to the morphology of the chromosomes, the material falls naturally into two groups, the one comprising the Dipnoi and Urodela, the other Anura.

(1) The first distinction between these groups is the size of chromosome. The first-mentioned group has immensely large chromosomes, being indeed among the largest known, while those of the second group, the Anurans, at the same stage are considerably smaller (Scheme 1).

(2) Moreover, the Dipnoi and Urodela, unlike Anura (Figs. 1—15), usually have a so-called hollow spindle (DARLINGTON, 1937).

(3) Another difference, which at first sight may not seem of much consequence, but which may give certain phylogenetically important suggestions, is that the chromosome lengths in Urodela and Dipnoi form a steadily falling curve, whereas for all the Anura examined there is a steep step approximately in the middle of the curve, possibly indicating an incipient division of the set into a group of large chromosomes and a group of small ones (*v.* Ch. I A).

(4) Also in regard to meiosis there are marked differences. With the exception of the family Discoglossidae (*v. infra*), Anura have throughout both major and minor spirals, whereas all the Urodela and Dipnoi examined have only the minor spiral developed.

It is true that some investigators (GALGANO, 1933; SATO, 1939 and, to judge by published pictures, also JANSSENS et WILLEMS, 1909, and CHAMPY, 1923) have found a mitotic appearance (= minor spirals only) in the chromosomes during the first division of meiosis, and a higher chiasma frequency than is normal in the Anurans among members of the family Discoglossidae, which taxonomically is one of the most primitive Anurans. (Cf. p. 263 ff.) It should be noted, however, that *Xenopus*, which, together with Pipidae, forms the second division of Opistocoela, corresponds in this respect with the other Anurans. It cannot therefore be taken for granted that the said feature in Discoglossidae is to be regarded as primary, thus bearing out SATO's (1939) view regarding a close phylogenetic connection between Anura and Urodela.

Considerable interest has been excited by SWINGLE's (1925) discovery that in *Rana catesbiana* the first meiosis in the larvae is marked by long slender bivalents »of Urodelian type» (SATO, 1939). IRIKI (1932 c), SAEZ *et al.* (1936), and SATO (1939), in accordance with HAECKEL's biogenetic rule, conclude from SWINGLE's discovery that Anura have developed from Urodela. But, even *if we accept the occurrence of this type of bivalents in the larvae of Rana catesbiana and in the Discoglossidae as a proof that the Anurans are derived from ancestors with single spirals during meiosis, it does not follow that those ancestors must necessarily have been Urodelians. On the contrary, this would serve to bear out the view maintained here that Anura, Urodela, and Dipnoi have common ancestors, probably with only a single spiral in the meiotic chromosomes.* These ancestors are presumably to be looked for among the Crossopterygii (DOLLO, 1896; JARVIK, 1942).

(5) There are also differences in the chiasma frequency. Anura in the larger chromosomes have a lower number of chiasmata, in the smaller ones a higher number than the corresponding chromosome sizes in Urodela and Dipnoi (Table 5). In the last-mentioned groups the chromosome length and chiasma frequency has been shown to be more closely correlated than in Anura, where — except in the shortest arms — the number of chiasmata is about one per arm (Table 5).

Thus in these respects: chromosome size, meiotic spiralization,

chiasma frequency, type of spindle, etc., the correspondence between the Urodelians and the Dipnoi is complete, whereas the Anurans show deviations. Also in regard to sex determination there appear to be certain differences between Anura and Urodela (Ch. III).

It thus seems to me evident that the distribution of the said features among Dipnoi, Urodela, and Anura indicates a closer relationship between Urodela and Dipnoi than between Anura and either of the other two groups.

From the viewpoint of comparative anatomy, the opinions expressed here seem to be well warranted. The phylogeny of the more primitive tetrapods has been studied in recent years by a number of, chiefly Swedish, investigators (WINTREBERT, 1922; HOLMGREN, 1933, 1939; SÄVE-SÖDERBERG, 1933, 1934, 1935, 1936; HERRE, 1935, and JARVIK, 1942). SÄVE-SÖDERBERG in particular (1933, pp. 114—118; 1934, pp. 5—6) has stressed the close relationship between the most primitive tetrapods, the Dipnoi and the Crossopterygii, thus confirming the views first advanced by DOLLO (1896), notably that these groups may be derived from common ancestors, probably the primitive Crossopterygii.

That the tetrapod group afterwards shows diverging lines of evolution and that the usual lumping together of Anura and Urodela to »Amphibia» is not phylogenetically warranted has also been pointed out by several of the said investigators, who have arrived at this conclusion by different lines: SÄVE-SÖDERBERG (1933, 1934, 1935, 1936) bases it on a study of the dermal bones in the skull (1935, p. 201: »*Amphibia* is a polyphyletic group and can not be regarded as a systematic unit»); HOLMGREN (1933, 1939) on the skeleton of the extremities; JARVIK (1942) on the skeletal components in the snout; HERRE (1935) and WINTREBERT (1922) on the different parts of the skeleton. In regard to the closer relationship between these different groups, however, there is a divergence of opinion. HOLMGREN considers that Anura, via the Stegocephalii, may be derived from certain Crossopterygii, whereas Urodela show a closer relationship with Dipnoi. This view is endorsed by SÄVE-SÖDERBERG and WINTREBERT and, as regards the connection of the Dipnoi with the Urodelians, also by HERRE. SÄVE-SÖDERBERG (1933, p. 168) states: »1. The Anura clearly belong to the group *Batrachomorpha* (*Ichtyostegalia—Labyrinthodontia—Phyllospondyli*). Their ancestors must have been very closely related to the *Labyrinthodontia* or actually belonging to that group» . . . and »3. The Urodeles fall definitely outside the evolutionary line, represented by the

Batrachomorpha (*Ichtyostegalia—Labyrinthodontia—Phyllospondyli—Anura*), and seem to be closely related to the Dipnoi». He further states (*loc. cit.*): »In recent Urodeles there are differences from the conditions in *Labyrinthodontia* and *Anura* . . . and striking similarities to Dipnoans (and partly also to Coelancanthids)».

Also WATSON derives *Anura* from *Labyrinthodontia* (1940, p. 229): »The order Phyllospondyli arose from the embolomerous Labyrinthodontia at an early period and its family Miobatrachidae gave origin to the Anura». He considers the derivation of the Urodeles to be more obscure.

JARVIK, on the other hand, has arrived at the conclusion (1942, p. 395) that: ». . . *Porolepiformes*[1] and *Urodeles* are closely related forms and that the Urodeles are derived from Porolepiformes[1] or at least from ancestors very closely akin to them». He adds (p. 551): ». . . The *Osteolepiformes*[1] are very closely related to the Anura and . . . the *Osteolepiformes*[1] include or at any event are very near to the ancestors of the Anura», and (p. 648): »The snout in *Dipnoi* is greatly modified and differs considerably from that in other forms». As regards the differences between the Dipnoans and the *Coelacanthidae*[1], however, JARVIK adds (p. 620): »It may be emphasized, however, that most of the differences are certainly due to the secondary modifications in the Dipnoans in correlation with the transformation of the dentition into tritoral plates . . .».

The cytological facts set forth in this paper are thus in correspondence with the results obtained by methods of comparative anatomy, *which is a clear indication that the cytological similarities and dissimilarities found between the different groups in this material have a real phylogenetic conclusiveness.* The cytological conditions correspond *well with the view that Anura, Urodela, and Dipnoi belong to different branches of the same large vertebrate stock.* They also agree well with the above cited view that *Anura nevertheless deviate from the two other groups.* In such circumstances it is perhaps not too bold to consider that *the surprisingly great cytological resemblances between the Dipnoans and the Urodeles have conclusive force in endeavour to determine the relationship between these groups. In my opinion, they strongly bear out the view* advanced by HOLMGREN (1933, 1939), SÄVE-

[1] JARVIK (*op. cit.*, p. 641) groups Crossopterygii as follows: Crossopterygii. I. Rhipidistia. A. *Porolepiformes.* B. *Osteolepiformes.* II. Actinistia. A. *Coelacanthiformes.*

SÖDERBERG (1933, 1934, 1936) and others *that the Dipnoans are closely related to the Urodeles.*

This view conflicts, in some measure, with that of JARVIK (1942). The cytological facts set forth here, however, are so strongly in favour of HOLMGREN's and SÄVE-SÖDERBERG's views that the latter must be considered to be well-grounded. It is thus interesting to find that *cytology can evidently be used for comparisons between different organisms from a phylogenetic point of view, even when those organisms are so remotely related as the vertebrates dealt with here.*

The *Osteolepiformes—Anura* line of development includes, according to JARVIK, also the Stegocephals and thus also reptiles (HOLMGREN, 1933, 1939; SÄVE-SÖDERBERG, 1934). The investigation reported here is not intended for the present to comprise Amniota, but, in order to ascertain whether the cytological peculiarities found in the more primitive Tetrapods also occur in the more developed forms, a study of *Emys* has also been made. I have deliberately selected a turtle, as Chelonia are apparently the now living form of reptile which most closely corresponds to the Stegocephals (VALLÉN, 1942). As previously shown (see OGUMA and MAKINO, 1937, for literature), the chromosome number in these reptiles is considerably higher than in Anura, being $2n = 50—64$, but most of the chromosomes are rod-shaped. In other respects, however, the correspondence is rather good (see Table 17), although Chelonia appear to have a well-developed sex chromosome mechanism (OGUMA, 1937). That *Emys* shows marked cytological resemblances to Anura is incontestable.

I have not yet had an opportunity to study the other reptile groups, but published pictures (RISLEY, 1936; NAKAMURA, 1935; se OGUMA and MAKINO, 1937, for other literature) indicate that the correspondence is not as good as in *Emys*. Thus the major spiral is missing in many forms, which has given IRIKI occasion for the following remark (1932 c, p. 103): »The discovery of horizontal V's of multiple rings in the first division of Urodela throws a new light on the phylogenetical relation to reptiles and mammals». NAKAMURA takes the same line (1935, p. 365): »From the phylogenetical point of view the ancestral reptiles are closely related to Urodeles». The resemblance between the chromosomes of reptiles and Urodeles, so far as I can see from published pictures, is very superficial and is confined to the absence of the major spiral in certain individual cases. At any rate, in my opinion the occurrence or absence of the major spiral alone is not such a fundamental feature as to warrant us in drawing such far-reaching conclusions, at variance

with all modern phylogenetic views, as IRIKI and NAKAMURA have done.

The previously mentioned step in the chromosome length curves in Anura (*v.* Ch. I A) may possibly represent an incipient division of the set into a group of large chromosomes and a group of small ones. Such a marked grouping has actually been found in most of the reptiles (MATTHEY, 1931; NAKAMURA, 1935; see OGUMA and MAKINO, 1937, for further literature). This, too, may perhaps be an indication of the phylogenetic relationship between the Anurans and the reptiles.

It is, indeed, astounding that these cytological features have evidently kept so constant during evolution that these groups, whose common ancestors must be sought in the Palaeozoic era, still show such striking and extensive resemblances. Urodela and Dipnoi, for example, can be cytologically distinguished only by the chromosome number, in which respect the difference between these groups is not more marked than between the members of different suborders of Urodela, such as the genera *Hynobius* and *Triturus*. It may be foreseen that cytological facts will play a considerable part in coming discussions concerning phylogenetic questions, not only as regards subspecies, species and families, but also in regard to larger systematic units. That cytological changes must play a very important rôle in phylogeny is evident, as also that most of these changes will consist of minute gene mutations (HUXLEY, 1942), which partly explains why the chromosome morphology shows such striking resemblances in these forms, despite their comparatively remote relationship.

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SUMMARY.

(1) The mitosis in all the forms examined is quite normal.

(2) The diploid chromosome numbers for 17 species have been determined. Three of these determinations are new. In three additional cases the figures given by previous authors have been corrected.

(3) The chromosome length at mitotic metaphase is considerable, being up to 30 microns.

(4) *Protopterus* and Urodela have larger chromosomes than Anura and *Emys*.

(5) All these forms (except the suborder of Urodeles, Cryptobranchoidea) have all their chromosomes distinctly two-armed.

(6) Anura and *Emys* are distinguished by a marked step in the chromosome length curve. There is a certain connection between the varying location of this step and chromosome number.

(7) As regards chromosome number and morphology, the Dipnoi appear to be a very uniform group.

(8) Urodela appear to be more heterogeneous. In this group two lines of development can be traced. The one, cytologically characterized by »fragmentations», leads from the Dipnoi through the Cryptobranchoidea; the other, cytologically characterized by »fusions», comprises the remaining suborders and may conceivably be derived from Cryptobranchoidea via Ambystomoidea and Salamandroidea to the final stage, which may possibly be Proteida.

(9) Anura are very uniform. The higher chromosome numbers, found among the more primitive forms, seem to point to »fusions» as an active factor in the phylogeny.

(10) The meiosis in these forms is quite normal.

(11) The zygotene-pachytene stages are characterized by a marked polarization of all the chromosome ends. This polarization seems, in some way, to be connected with the centromere as it disappears after colchicine treatment.

(12) The major spiral is missing in Dipnoi and Urodela, but occurs

in Anura (except for the family Discoglossidae and certain anomalies in other forms) as well as in *Emys*.

(13) The distal localization of the chiasmata in these species is due to the initiation of the pairing at the distal ends, the commencement of the division at the centromere and a restricted time limit between pairing and division.

(14) In regard to chiasma frequency, Urodela are rather heterogeneous, whereas Anura are very homogeneous. Urodela, however, have throughout a higher frequency than Anura, and the difference in this respect is immense even in chromosomes of equal length.

(15) The connection between chiasma frequency and length of chromosome and chromosome arms has been thoroughly studied. In *Protopterus* and Urodela the chiasma frequency is correlated to the chromosome length; this is not the case in Anura, except in the smallest chromosomes.

(16) The chiasma frequency in triploid Anura and Urodela corresponds with $^2/_3$ of the values found in the closely related diploid species, and the difference from previously studied triploids is a function of the low chiasma frequency in the forms dealt with here.

(17) The temperature affects the chiasma frequency, though indirectly, by modifying the time limit. Certain data have been adduced, which point to some of the ways in which the chiasma frequency is affected by temperature, namely:

(a) The viscosity of the plasma, which is dependent on the temperature according to a W-shaped curve (HEILBRUNN, 1928).

(b) The physiological, chemical and physical reactions which take place during the division of the chromosomes at diplotene. The speed of these reactions should depend on the temperature similarly as other such reactions (BĚLEHRÁDEK, 1935).

(c) The size of the nucleus, which is dependent on the temperature according to a U-shaped curve (HARTMANN, 1919). The size of the nucleus, in turn, is a function, not of the temperature direct, but of the other factors, such as osmosis, which are dependent on temperature (BĚLEHRÁDEK, *op. cit.*, pp. 94 ff. and 179, for literature).

(18) As the temperature, in different ways, can simultaneously restrict and extend the time limit, the effect of variations in temperature is very complex. In each case it includes the following components:

(a) 17a above.
(b) 17b above.
(c) 17c above.
(d) The reaction of the species or individual to the influences referred to under 17a—c.
(e) Moreover, the heterochromatin seems to be more sensitive to temperature effects than the euchromatin (MATHER, 1938 b).

(19) For different organisms, the peaks and throughs in the curves according to 17a—c are situated at different temperatures, depending, *inter alia*, on the temperature in their normal environment, etc.

(20) The chiasmata show a high degree of terminalization in all species examined. There is a marked difference between *Protopterus* and Urodela, on the one hand, with a terminalization coefficient of about 0,5 at metaphase and Anura and *Emys*, on the other, with a terminalization coefficient of 0,89—0,99 at metaphase.

(21) The degree of terminalization of the chiasmata and the spiralization of the chromosomes is inversely proportional to the temperature; this action of temperature is also indirect and may be derived from the influence of temperature upon the rapidity of the meiosis. In a rapid division (heat) the chromosomes have not time enough for spiralization and terminalization of their chiasmata; in a slow division (cold) the spiralization and terminalization become very strong.

(22) Nucleic acid starvation (DARLINGTON and LA COUR, 1940; CALLAN, 1942) can be produced by cold. Fasting has a similar effect. In the forms dealt with here, the heterochromatic segments which are brought to light by the said treatment are mainly proximal.

(23) By colchicine treatment, diploid and tetraploid spermatozoa can be developed in *Triturus*.

(24) Polyploidy has played no part in the phylogeny of the here treated forms. The reasons for this lie in the facts that such autopolyploids as occasionally occur cannot produce balanced offspring by sexual reproduction (multivalents!) and that asexual propagation does not occur in vertebrates; that, in view of the special pairing instincts of the animals, hybrids are rare; and that such hybrids cannot, like plants, produce allopolyploids, owing to the different processes of growth in plants and animals.

(25) The polyploids in Anura and Urodela do not show any greater size or vitality than the diploids. However, the cells are greater in the polyploids.

(26) The chromatids which appear during the prophase and metaphase of mitosis and which separate at anaphase, must have been produced by the longitudinal division of their mother chromosome in the immediately preceding resting stage.

(27) Pictures of doubleness in the mitotic chromatids, obtained after alkaline treatment, are produced by the treatment itself, possibly in the way that the pH of the nucleus is thus raised to that of the resting stage; the nucleus thus artificially reverts to a resting stage, with the chromosome division and despiralization phenomena characteristic of that stage.

(28) The plane along which the chromosome division takes place is screw-shaped, whence the daughter chromatids from the moment of »birth» are relational coiled.

(29) The marked relational coiling which can be observed at metaphase after KOH-treatment, is produced in another way. On the despiralization of the chromatids, the ends rotate and get coiled round one another.

(30) In two cases the spiral at the mitotic metaphase has been drawn out by mechanical means and has thus been made accessible for observation.

(31) Despiralization with KOH facilitated the examination of the major spiral during the second division of meiosis. Changes in direction of spiralization have been observed.

(32) The sex chromosomes in Anura and Urodela are not cytologically recognizable.

(33) The cytological findings conduce to support the view as to the phylogenetic relationship between Dipnoi, Urodela, Anura, and Chelonia.

(34) There are nevertheless such striking similarities between the Dipnoi and Urodela, on the one hand, as well as between Anura and Chelonia, on the other, and such marked differences between the two groups themselves, that the usual unification of Anura and Urodela as »Amphibia» cannot be defended. The cytological findings, instead, conduce to bear out certain modern views in comparative anatomy, according to which Urodela are closely related to the Dipnoi, whereas Anura and Chelonia are derived from Stegocephalia. Both these lines of evolution can then be traced back to common ancestors in the Crossopterygii.

EXPLANATION OF PLATES I—III.

Plate I.

Fig. 1. Mitotic metaphase of *Protopterus annectens.* — Fig. 2. Mitotic metaphase of *Protopterus annectens.* — Fig. 3. Early mitotic prophase of *Bufo vulgaris* showing relic spirals. — Fig. 4. Mitotic metaphase of *Ambystoma mexicana* treated with 0,1 % KOH, 5 minutes. Strong relational coiling between chromatids. — Microphotographs.

Plate II.

Fig. 1. Diplotene of *Triturus cristatus.* — Fig. 2. M^I of *Triturus cristatus.* — Fig. 3. M^I of *Bufo calamita.* — Fig. 4. M^I of *Triturus vulgaris.* (600 ×.) — Fig. 5. Late diakinesis of *Triturus vulgaris.* (600 ×.) — Fig. 6. M^I of *Protopterus annectens.* — Fig. 7. M_I of *Xenopus laevis.* — Microphotographs.

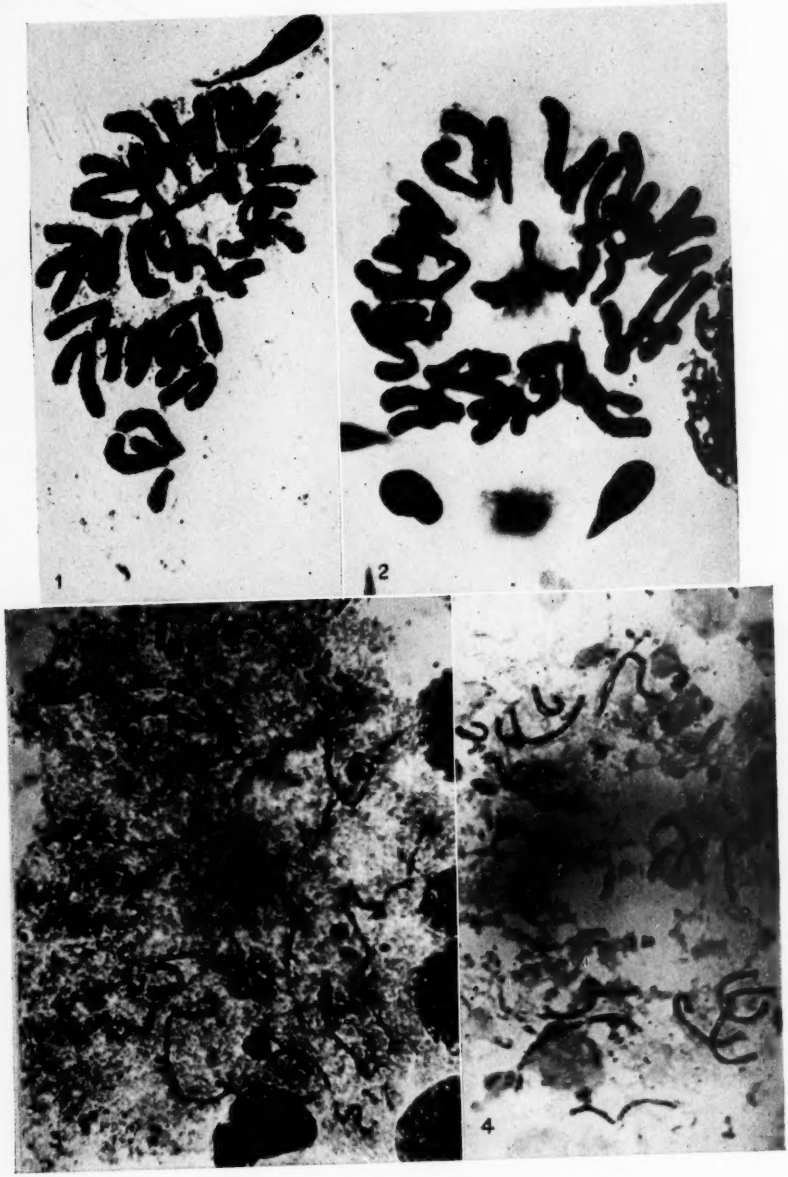
Plate III.

Fig. 1. Diakinesis of *Rana arvalis.* — Fig. 2. *Triturus cristatus.* Mitotic telophase with fragment after cold treatment. — Fig. 3. *Triturus cristatus.* Meiosis. Colchicine treatment. — Fig. 4. *Triturus cristatus.* Meiosis. Colchicine treatment. — Fig. 5. M^{II} of *Triturus cristatus.* (600 ×.) — Fig. 6. M^I of *Emys orbicularis.* — Fig. 7. Mitotic spiral of *Ambystoma mexicana.* (Cf. text and Fig. 40.) — Fig. 8. *Ambystoma mexicana.* Mitotic metaphase treated with 0,1 % KOH, 5 minutes. The chromatids in the middle show doubleness. Cf. Fig. 43. — Fig. 9. *Ambystoma mexicana.* Mitotic metaphase treated with 0,1 % KOH, 5 minutes. The left chromatid of the chromosome in the centre shows doubleness. Cf. Fig. 44. — Microphotographs.

LITERATURE CITED.

(Papers only read in abstract are marked by an asterisk.)

1. AGAR, E. E. 1911. The spermatogenesis of *Lepidosiren paradoxa.* — Quart. Journ. Micr. Sci. 57: 1—44.
2. — 1912. Transverse segmentation and internal differentiation of chromosomes. — Quart. Journ. Micr. Sci. 58: 285—298.
3. AISIMA, T. 1940. Studies of mitosis and meiosis in comparison. II. Chromosome structure in the spiral stage of anaphase in mitosis as revealed by means of a maceration method. — Cytologia 11: 429—435.
4. ATWOOD, S. 1937. The nature of the last premeiotic mitosis and its relation to meiosis in *Gaillardia.* — La Cellule 46: 391—406.
5. BARBER, H. N. 1939. The suppression of meiosis and the origin of diplochromosomes. — Proc. Roy. Soc. Lond. Ser. B. 128: 170—185.
6. — 1941. Chromosome behaviour in *Uvularia.* — Journ. of Gen. 42: 223—257.
7. BECCARI, N. 1926. Le nombre des chromosomes dans les cellules génitales de *Bufo viridis.* — Ann. Anat. Path. 3: 841.
8. BĚLAŘ, K. 1929. Beiträge zur Kausalanalyse der Mitose. III. Untersuchungen an den Staubfäden, Haarzellen und Blattmeristemzellen von *Tradescantia virginica.* — Zeitschr. f. Zellforsch. u. mikr. Anat. 10: 73—134.
9. BĚLEHRÁDEK, J. 1935. Temperature and living matter. — Protoplasma-Monographien, Bd. VIII.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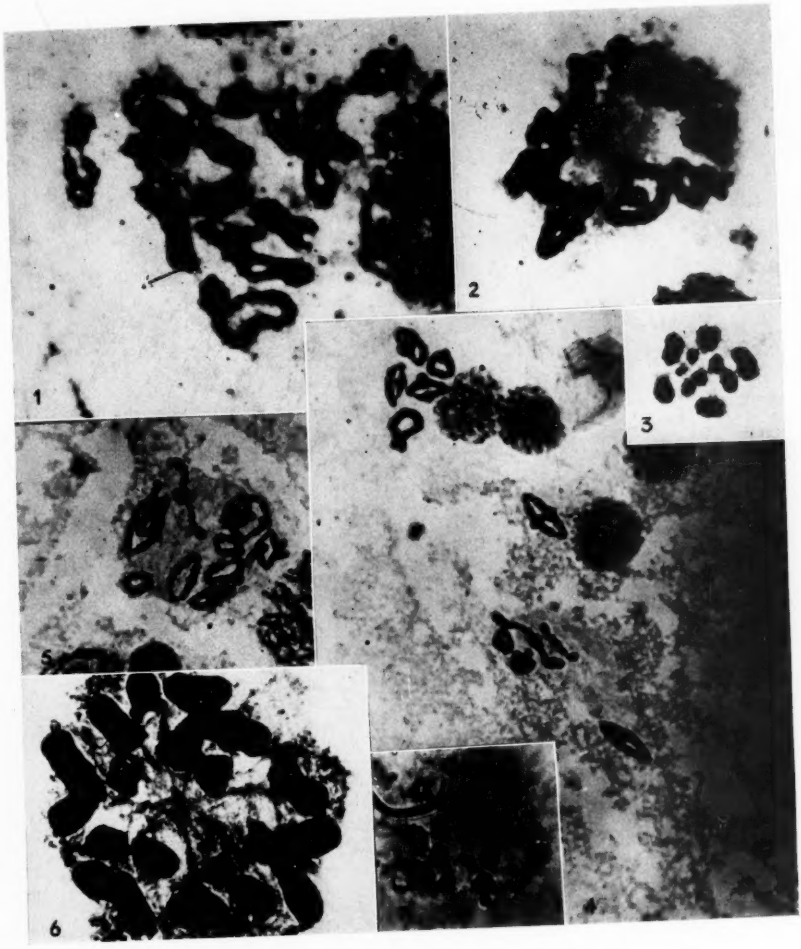
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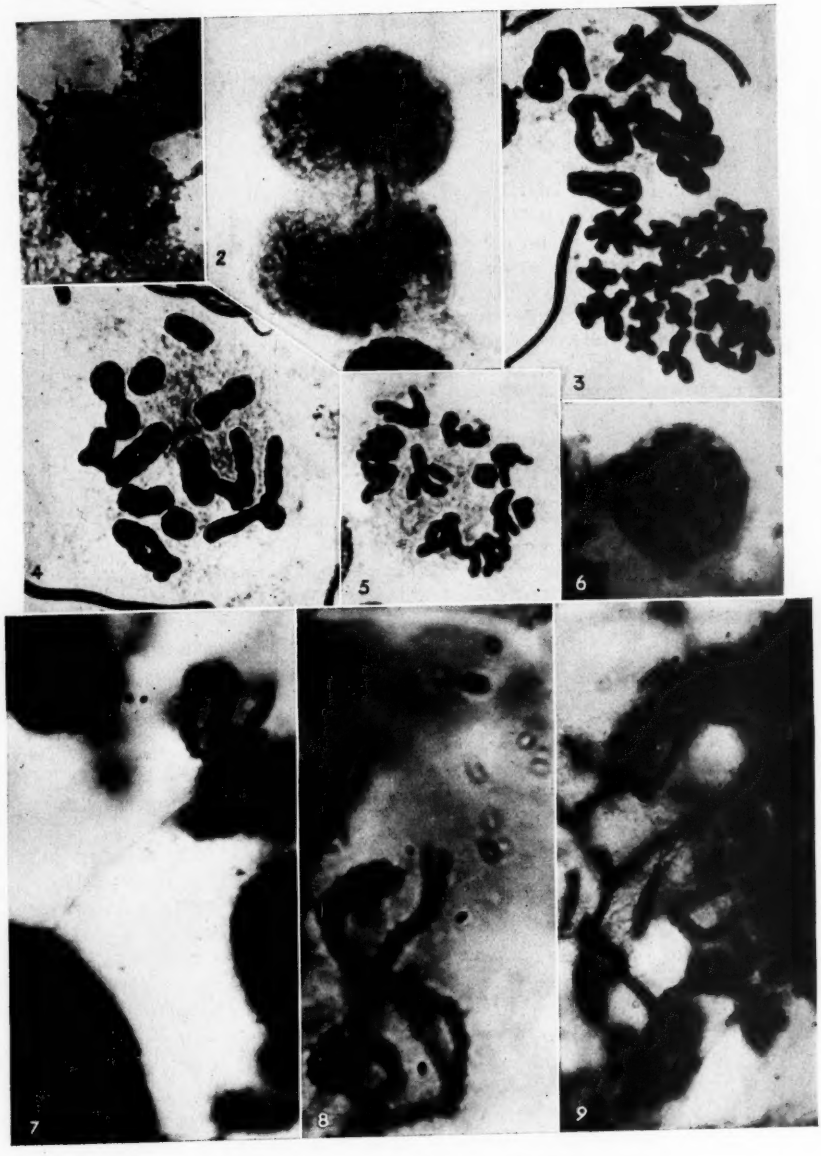
2
3
4
5
6
7
8
9

10. BENNETT, E. S. 1938. The origin and behaviour of chiasmata. XIV. *Fritillaria chitralensis*. — Cytologia 8: 443—451.
11. BONNIER, G. and TEDIN, O. 1940. Biologisk variationsanalys. — Stockholm.
12. BOOST, C. und LUDWIG, G. 1939. Über die Häufigkeit mehrfacher Chiasmen und ihre Beziehung zu einer gerichteten Chiasmabildung. — Chromosoma 1: 300—309.
13. BORGENSTAM, E. 1922. Zur Zytologie der Gattung *Syringa*. — Arkiv f. Botanik, Bd. 17, No. 15.
14. BOVERI, T. 1909. Die Blastomerenkerne von *Ascaris megalocephala* und die Theorie der Chromosomenindividualität. — Arch. f. Zellforsch. 3: 181—268.
15. BRYDEN, W. 1935. Some observations upon the mitotic and meiotic divisions in the Wistar rat. I. The effect of changes in temperature. — Cytologia 6: 300—307.
16. BUSHNELL, R. J., BUSHNELL, E. P., and PARKER, M. V. 1939. A chromosome study of five members of the family Hylidae. — Journ. Tennessee Acad. Sci. 14: 209—215. (Biol. Abstr. 1939, No. 10722.) (*).
17. BÖÖK, J. A. 1940. Triploidy in *Triton taeniatus* LAUR. — Hereditas XXVI: 107—114.
18. — 1941. Induction of haploidy in a cold treatment experiment with egg-cells of the salamander *Triton taeniatus*. — Kungl. Fysiogr. Sällsk. i Lund Förhandl. Bd. 11, No. 12: 1—16.
19. — 1943. Low temperature and cleavage in *Triton taeniatus*. — Hereditas XXIX: 195—197.
20. — 1945. Cytological studies in *Triton*. — Hereditas XXXI: 177—220.
21. CALLAN, H. G. 1942. Heterochromatin in *Triton*. — Proc. Roy. Soc. London, Ser. B. 130: 324—335.
22. CARNOY, J. B. et LEBRUN, H. 1899. La vésicule germinative et les globules polaires chez les Batrachiens. — La Cellule 16: 299—401.
23. CARRICK, R. 1934. The spermatogenesis of the Axolotl (*Ambystoma tigrinum*). — Trans. Roy. Soc. Edinb. 58: 63—74.
24. CHAMPY, G. 1923. La spermatogénèse chez *Discoglossus pictus* (OTTH.). — Arch. Zool. exp. et gén. 62: 1—52.
25. COLEMAN, L. C. 1940. The structure of homotypic and somatic chromosomes. — Am. Journ. of Bot. 27: 683—686.
26. CREIGHTON, M. 1938. Chromosome structure in *Amblystoma punctatum*. — Cytologia 8: 497—504.
27. CREW, F. 1921. Sex reversal in frogs and toads. — Journ. of Gen. 11: 141—181.
28. DALCQ, A. 1930. La formule chromosomiale chez la grenouille. — Ann. Soc. Roy. med. nat. Brux. 1930: 15—21.
29. DARLINGTON, C. D. 1936. Crossing-over and its mechanical relationships in *Chorthippus* and *Stauroderus*. — Journ. of Gen. 33: 465—500.
30. — 1937. Recent advances in cytology. 2nd ed. — London.
31. — 1940 a. The prime variables of meiosis. — Biol. Rev. 15: 307—322.
32. — 1940 b. The causal sequence of meiosis. II. Contact points and crossing-over potentials in a triploid *Fritillaria*. — Journ. of Gen. 41: 35—48.
33. DARLINGTON, C. D. and DARK, S. O. S. 1932. The origin and behaviour of chiasmata. II. *Stenobothrus parallelus*. — Cytologia 3: 169—185.

34. DARLINGTON, C. D. and LA COUR, L. 1940. Nucleic acid starvation of *Trillium*. — Journ. of Gen. 40: 185—213.
35. — and — 1942. The handling of chromosomes. — London.
36. DARLINGTON, C. D. and MATHER, K. 1932. The origin and behaviour of chiasmata. III. Triploid *Tulipa*. — Cytologia 4: 1—15.
37. DEARING, W. H. 1934. The material continuity and individuality of the somatic chromosomes of *Amblystoma tigrinum*, with special reference to the nucleolus as a chromosomal component. — Journ. of Morph. 56: 157—174.
38. DOLLO, L. 1896. Sur la philogénie des Dipneustes. — Bull. Soc. Belg. Géol. Bruxelles, 9: 79—128.
39. DÜRKEN, B. 1938. Über die Keimdrüsen und die Chromosomen der Artbastarde *Rana arvalis* NILSS. × *Rana fusca* RÖS. — Zeitschr. f. ind. Abst.- u. Vererbungslehre 74: 331—353.
40. ELVERS, I. 1943. On an application of the electron microscope to plant cytology. — Acta Horti Bergiani, Bd. 13, No. 5: 149—245.
41. FANKHAUSER, G. 1934. Cytologic studies on egg fragments of the salamander *Triton*. V. Chromosome number and chromosome individuality in the cleavage mitoses of merogonic fragments. — Journ. Exp. Zool. 68: 1—57.
42. — 1938 a. Triploidy in the newt, *Triturus viridescens*. — Proc. Amer. Phil. Soc. 79: 715—739.
43. — 1938 b. Sex differentiation in triploid salamanders (*Triturus viridescens*). — Anat. Rec. 72, Suppl. 70.
44. — 1939 a. The effects of triploidy on cell size and organ size in salamanders. — Genetics 24: 71.
45. — 1939 b. Polyploidy in the salamander, *Eurycea bislineata*. — Journ. of Hered. 30: 379—388.
46. — 1940. Sex differentiation in triploid newts (*Triturus viridescens*). — Anat. Rec. 77: 227—239.
47. — 1941 a. The effects of pentaploidy on development in the newt, *Triturus viridescens*. — Genetics 26: 150.
48. — 1941 b. Cell size, organ and body size in triploid newts (*Triturus viridescens*). — Journ. of Morph. 68: 161—174.
49. FANKHAUSER, G. and GRIFFITHS, R. B. 1939. Induction of triploidy and haploidy in the newt, *Triturus viridescens*, by cold treatment of unsegmented eggs. — Proc. Nat. Acad. Sci. Wash. 25: 233—238.
50. FANKHAUSER, G. and KAYLOR, C. T. 1935. Chromosome numbers in androgenetic embryos of *Triturus viridescens*. — Anat. Rec. 64, Suppl.: 41—42.
51. FRANKEL, O. H. 1940. The causal sequence of meiosis. I. Chiasma formation and the order of pairing in *Fritillaria*. — Journ. of Gen. 41: 9—34.
52. GALGANO, M. 1933. Evolutione dei spermatociti di I ordine e cromosomi pseudosessuali in alcune specie di anfibi. — Arch. Ital. Anat. Embr. 32: 171—200.
53. — 1941. La variazione dei chiasmi nei maschi e negli intersessuati di »*Rana esculenta*» L. — Arch. Ital. Anat. Embr. 56: 127—165.
54. GEITLER, L. 1935. Der Spiralbau somatischer Chromosomen. — Zeitschr. f. Zellforsch. u. mikr. Anat. 23: 514—521.
55. — 1938. Chromosomenbau. — Protoplasma-Monographien, Bd. 14.

56. GEITLER, L. 1940. Neue Ergebnisse und Probleme auf dem Gebiet des Chromosomenbaues. — Naturwiss. 28: 649—656.
57. GELEI, J. 1921. Weitere Studien über die Oogenese des *Dendrocoelum lacteum*. — Arch. f. Zellforsch. 16: 88—169, 299—370.
58. GOLDSCHMIDT, R. 1920. Kleine Beobachtungen und Idéen zur Zellenlehre. II. Die Spermatogenese eines parthenogenetischen Frosches nebst Bemerkungen zur Frage, welches Geschlecht bei den Amphibien das heterozygotische ist. — Arch. f. Zellforsch. 15: 283—290.
59. GRIFFITHS, R. B. 1940. Triploidy in *Triturus viridescens*, induced by exposure of eggs to low temperature. — Anat. Rec. 76, Suppl. 26—27.
60. — 1941. Triploidy (and haploidy) in the newt, *Triturus viridescens*, induced by refrigeration of fertilized eggs. — Genetics 26: 58—88.
61. HAGA, T. 1937. Karyologic polymorphism in *Paris hexaphylla* CHAM. with special reference to its origin and to the meiotic chromosome behaviour. — Cytologia, FUJII Jub. Vol.: 681—700.
62. HARTMANN, O. 1919. Über den Einfluss der Temperatur auf Plasma, Kern und Nucleolus und zytologische Gleichgewichtszustände. — Arch. f. Zellforsch. 15: 177—248.
63. HEILBORN, O. 1930. Temperatur und Chromosomenkonjugation. — Sv. Bot. Tidskr. 24: 12—25.
64. HEILBRUNN, L. V. 1928. The colloid chemistry of protoplasm. — Protoplasma-Monographien, Bd. 1.
65. HERRE, W. 1935. Die Schwanzlurche der mitteleocänen Braunkohle des Geiseltales und die Phylogenie der Urodelen unter Einschluss der fossilen Formen. — Zoologica 33; Heft 87: 1—85.
66. HERTWIG, G. 1918. Kreuzungsversuche an Amphibia. I. Wahre und falsche Bastarde. — Arch. mikr. Anat. 91; 2: 203—271.
67. HERTWIG, G. und HERTWIG, P. 1920. Triploide Froschlarven. — Arch. mikr. Anat. 94: 34—54.
68. HERTWIG, P. 1936. Artbastarde bei Tieren. — Handb. d. Vererbungswiss., Bd. II.
69. HIRAOKA, T. 1941. A contribution to the study of the origin of the »bouquet» and its formation. — Cytologia 11: 483—492.
70. HOLMGREN, N. 1933. On the origin of the tetrapod limb. — Acta Zoologica 14: 185—295.
71. — 1939. Contribution to the question of the origin of the tetrapod limb. — Acta Zoologica 20: 89—124.
72. HRUBY, K. 1934. Über die Chromosomenstruktur bei infraroten Strahlen. — Planta 22: 685—691.
73. HUSKINS, C. L. 1937. The internal structure of chromosomes — a statement of opinion. — Cytologia, FUJII Jub. Vol.: 1015—1022.
74. HUSKINS, C. L. and NEWCOMBE, H. B. 1941. An analysis of chiasma pairs showing chromatid interference in *Trillium erectum* L. — Genetics 26: 100—127.
75. HUXLEY, J. 1942. Evolution — the modern synthesis. — Oxford.
76. IRIKI, S. 1928. On the chromosomes of Amphibia. — Zool. Mag. 40: 491. (*).
77. — 1929. On the chromosomes of *Bufo bufo japonicus*. — Zool. Mag. 41: 491. (*).

78. IRIKI, S. 1930. Studies on Amphibian chromosomes. I. On the chromosomes of *Hyla arborea japonica* GUENTHER. — M. C. S. Kyoto Imp. Univ. Ser. B. 5: 1—19.

79. — 1932 a. Studies on Amphibian chromosomes. IV. On the chromosomes of *Rana rugosa* and *Rana nigromaculata*. — Sci. Rep. Tokyo Bunr. Daig. Sec. B. 1: 61—72.

80. — 1932 b. Studies on Amphibian chromosomes. V. Explanation of the sex chromosome type of *Hyla arborea japonica* from the standpoint of spiral structure. — Sci. Rep. Tokyo Bunr. Daig. Sec. B. 1: 73—80.

81. — 1932 c. Studies on Amphibian chromosomes. VII. *Megalobatrachus japonicus*. — Sci. Rep. Tokyo Bunr. Daig. Sec. B. 1: 91—104.

82. JANSSENS, F. A. 1900. Rapprochement entre les cinéses polliniques et les cinéses sexuelles dans le testicule des Tritons. — Anat. Anz. 17: 520—524.

83. — 1901. La spermatogénèse chez les Tritons. — La Cellule 19: 5—116.

84. — 1902. Die Spermatogenese bei den Tritonen nebst einigen Bemerkungen über die Analogie zwischen chemischer und physikalischer Tätigkeit der Zelle. — Anat. Anz. 21: 129—138.

85. — 1904. Das chromatische Element während der Entwicklung des Oocyts des Triton — Anat. Anz. 24: 648—651.

86. — 1909. Spermatogénèse dans les Batrachiens. V. La théorie de la chiasmatypie, nouvelle interpretation des cinéses de maturation. — La Cellule 25: 387—411.

87. JANSSENS, F. A. et WILLEMS, J. 1909. La spermatogénèse dans l'*Alytes obstetricans*. — La Cellule 25: 151—173.

88. JARVIK, E. 1942. On the structure of the snout of Crossopterygians and lower Gnathostomes in general. — Zool. Bidr. fr. Uppsala, Bd. 21: 235—675.

89. JEFFREY, E. C. 1937. The somatic chromosomes of *Trillium*. — Cytologia, FUJII Jub. Vol.: 857—866.

90. JOLLY, J. 1904. Recherches experimentales sur la division indirecte des globules rouges. — Arch. Anat. micr. 6: 455—629.

91. KAWAMURA, T. 1939 a. Artificial parthenogenesis in the frog. I. Chromosome numbers and their relation to the cleavage histories. — Journ. of Sci. Hiroshima Univ. Ser. B. Div. 1, 6: 115—218.

92. — 1939 b. Artificial parthenogenesis in the frog. II. The sex of parthenogenetic frogs. — Journ. of Sci. Hiroshima Univ. Ser. B. Div. 1,7: 39—86.

93. KIHARA, H. 1927. Über das Verhalten der »end-to-end» gebundenen Chromosomen von *Rumex acetosella* und *Oenothera biennis* während der heterotypischen Kernteilung. Beitrag zur Frage der Para- und Metasyndese. — Jahrb. wiss. Bot. 66: 429—460.

94. KING, H. D. 1912. Dimorphism in the spermatozoa of *Necturus maculosus*. — Anat. Rec. 6: 405—411.

95. KLINGSTEDT, H. 1936. On some tetraploid spermatocytes in *Chrysochraon dispar*. (Orth.) — Mem. Soc. Fauna et Flora Fenn. 12—13: 194—209.

96. KOLLER, P. C. 1936. The genetical and mechanical properties of the sex chromosomes. II. Marsupials. — Journ. of Gen. 32: 79—102.

97. — 1937. The genetical and mechanical properties of the sex chromosomes. III. Man. — Proc. Roy. Soc. Edinb. 57: 194—214.

98. KUWADA, Y. 1938. Behaviour of chromonemata in mitosis. VII. A chromosome study by the artificial uncoiling method of the chromonema spirals. — Cytologia 9: 17—22.
99. — 1939. Chromosome structure. A critical review. — Cytologia 10: 213—256.
100. KUWADA, Y. and NAKAMURA, T. 1934. Artificial unravelling of coiled chromonemata. — Cytologia 5: 244—247.
101. LANTZ, L. A. 1934. Molchbastarde. — Bl. f. Aquar. und Terrar.-Kunde, 45: 23—32.
102. LEVAN, A. 1936. Zytologische Studien an *Allium Schoenoprasum*. — Hereditas XXII: 1—128.
103. — 1938. The effect of colchicine on root mitoses in *Allium*. — Hereditas XXIV: 471—486.
104. — 1939. The effect of colchicine on meiosis in *Allium*. — Hereditas XXV: 9—26.
105. LOEB, J. 1918. Further experiments on the sex of parthenogenetic frogs. — Proc. Nat. Acad. Sci. Wash. 4: 60—62.
106. LÖVE, A. 1944. Cytogenetic studies on *Rumex* subgenus *Acetosella*. — Hereditas XXX: 1—136.
107. MCCLUNG, C. E. 1905. The chromosome complex of Orthopteran spermatocytes. — Biol. Bull. 9: 304—340.
108. — 1914. A comparative study of the chromosomes in Orthopteran spermatogenesis. — Journ. of Morph. 25: 651—749.
109. MCGREGOR, J. H. 1899. The spermatogenesis of *Amphiuma*. — Journ. of Morph. 15, Suppl.: 57—104.
110. MAKINO, S. 1932 a. Notes on the chromosomes of *Rana temporaria* L. and *Bufo sachalinensis* (NIKOLSKI). — Proc. Imp. Acad. Tokyo, 8: 23—24.
111. — 1932 b. Notes on the chromosomes of *Rhacophorus schlegelii schlegelii* GUENTHER. — Proc. Imp. Acad. Tokyo, 8: 24—26.
112. — 1932 c. The chromosome numbers in some salamanders from northern Japan. — J. F. S. Hokkaido Imp. Univ. Ser. VI, 2: 97—108.
113. — 1934. The chromosomes of *Hynobius leechii* and *H. nebulosus*. — Trans. Sapporo Nat. Hist. Soc. 13: 351—354.
114. — 1935 a. The chromosomes of *Hynobius dunni* and *H. kimurai*. — Jap. Journ. of Gen. 10: 243—244.
115. — 1935 b. The chromosomes of *Cryptobranchus allegheniensis*. — Journ. of Morph. 58: 573—584.
116. — 1939. The chromosomes of the carp, *Cyprinus carpio*. — Cytologia 9: 430—440.
117. MATHER, K. 1936. The determination of position in crossing over. I. *Drosophila melanogaster*. — Journ. of Gen. 33: 207—235.
118. — 1937. The determination of position in crossing over. II. The chromosome length—chiasma frequency relation. Cytologia, FUJII Jub. Vol.: 514—526.
119. — 1938 a. Crossing over. — Biol. Rev. 13: 252—292.
120. — 1938 b. Crossing-over and heterochromatin in the X chromosome of *Drosophila melanogaster*. — Genetics 24: 413—435.
121. MATHER, K. and STONE, H. A. 1933. The effect of x-radiation upon somatic chromosomes. — Journ. of Gen. 28: 1—24.

122. MATSUURA, H. 1937. Chromosome studies in *Trillium kamtschaticum* PALL. V. Abnormal divisions due to high temperature. — Cytologia, FUJII Jub. Vol.: 20—34.

123. — 1941. Chromosome studies in *Trillium kamtschaticum* PALL. XV. A contribution to the present status of knowledge on the mechanism of chromosome coiling. — Cytologia 11: 407—428.

124. MATTHEY, R. 1931. Chromosomes de Reptiles, Sauriens, Ophidiens, Chéloniens. L'évolution de la formule chromosomiale chez les Sauriens. — Rev. Suisse Zool. 38: 117—183.

125. MEEK, C. F. U. 1913. A metrical analysis of chromosome complex, showing correlation of evolutionary development and chromatin thread-width throughout the animal kingdom. — Phil. Trans. Roy. Soc. London, 23 B: 1—74.

126. MINOUCHI, O. and IRIKI, S. 1931. Studies on Amphibian chromosomes. 2. On the chromosomes of *Bufo bufo japonicus schlegelii*. — M. C. S. Kyoto Imp. Univ. Ser. B. 6: 39—43.

127. MULLER, H. J. 1916. The mechanism of crossing over. II. — Amer. Nat. 50: 193—224, 284—305, 350—366, 421—434.

128. — 1925. Why polyploidy is rarer in animals than in plants. — Amer. Nat. 59: 346—353.

129. MÜNTZING, A. 1936. The evolutionary significance of autopolyploidy. — Hereditas XXI: 263—378.

130. NAKAMURA, K. 1935. Studies on reptilian chromosomes. — M. C. S. Kyoto Imp. Univ. Ser. B. 10: 341—406.

131. NAKAMURA, M. 1936. Experimental and cytological studies in the unstability of the meiotic divisions of the pollen mother cells of *Impatiens balsamina* L. caused by the effect of high air temperature. — Mem. Fac. Sci. and Agr. Taihoku Imp. Univ. 12: 121—183.

132. NEBEL, B. R. 1939. Chromosome structure. — Bot. Rev. 5: 563—626.

133. NEWCOMBE, H. B. 1942. The action of X-rays on the cell. 1. The chromosome variable. — Journ. of Gen. 43: 145—171.

134. NOBLE, G. K. 1931. The biology of the Amphibia. — New York and London.

135. OEHLKERS, F. 1935. Untersuchungen zur Physiologie der Meiosis. I. — Zeitschr. f. Bot. 29: 1—53.

136. — 1936. Untersuchungen zur Physiologie der Meiosis. III. — Zeitschr. f. Bot. 30: 253—276.

137. OGUMA, K. 1937. The chromosomes of the soft shelled turtle, *Amyda japonica*, as additional proof of female heterogamety in the Reptilia. — Journ. of Gen. 34: 247—265.

138. OGUMA, K. and MAKINO, S. 1937. A new list of the chromosome numbers in Vertebrata (March, 1937). — Journ. Fac. Sci. Imp. Univ. Sapporo, Ser. 6, 5: 297—356.

139. PARMENTER, C. L. 1919. Chromosome number and pair in somatic mitoses of *Ambystoma tigrinum*. — Journ. of Morph. 33: 169—226.

140. — 1920. The chromosomes of parthenogenetic frogs. — Journ. of gen. Physiol. 2: 205—206.

141. — 1925. The chromosomes of parthenogenetic frogs and tadpoles. — Journ. of gen. Physiol. 8: 1—20.

142. PARMENTER, C. L. 1933. Haploid, diploid, triploid and tetraploid chromosome numbers and their origin in parthenogenetically developed larvae and frogs of *Rana pipiens* and *Rana palustris*. — Journ. exp. Zool. 66: 409—453.

143. PLOUGH, H. H. 1917. The effect of temperature on crossing over in *Drosophila*. — Journ. exp. Zool. 24: 147—209.

144. POLIAKOWA, T. F. 1940. Effect of high and low temperature upon chiasma formation in *Allium cepa* L. — C. R. Doklady Acad. Sci. U.R.S.S. 27: 594—597.

145. POSKA-TEISS, L. 1933. Spermatogonien von *Bufo vulgaris* LAUR. und ihr Vergleich mit larvalen somatischen Zellen desselben Tieres. — Zeitschr. f. Zellforsch. und mikr. Anat. 17: 347—419.

146. PROKOFIEWA, A. 1935. On the chromosome morphology of certain Amphibia. — Cytologia 6: 148—164.

147. PÄTAU, K. 1940. »The pairing coefficient». — Proc. 7th int. genet. Congr. Edinb. 1939.

148. — 1941. Cytologischer Nachweis einer positiven Interferenz über das Centromer. — Chromosoma 2: 36—63.

149. RABL, C. 1885. Über Zellteilung. — Morph. Jahrb. 10: 124—330.

150. RILEY, H. P. 1936. The effect of X-rays on the chromosomes of *Tradescantia gigantea*. — Cytologia 7: 131—142.

151. RISLEY, P. L. 1936. The chromosomes of the male musk turtle, *Sternotherus odoratus*. — Cytologia 7: 232—241.

152. ROBERTSON, W. R. 1916. Chromosome studies. — Journ. of Morph. 27: 179—280.

153. SAEZ, F. A., ROJAS, P. und DE ROBERTIS, E. 1936. Untersuchungen über die Geschlechtszellen der Amphibien (Anuren). I. Der meiotische Prozess bei *Bufo arenarium*. — Zeitschr. f. Zellforsch. u. mikr. Anat. 24: 727—777.

154. SAKAMURA, T. 1916. Über die Beeinflussung der Zell- und Kernteilung von der Chloralisierung mit besonderer Rücksicht auf das Verhalten der Chromosomen. — Bot. Mag. 30: 509.

155. SATO, I. 1934. On the chromosomes of *Rana limnocharis* and *Polypedates buergeri*. — Zool. Mag. 46: 98—104.

156. — 1936 a. On the chromosomes of some hynobiid salamanders from southern Japan. — Journ. of Sci. Hiroshima Univ. Ser. B, 4: 143—154.

157. — 1936 b. Notes on the chromosomes of *Cacopoides tornieri* and *Bufo raddei*. — Zool. Mag. 48: 958—960.

158. — 1939. Of the chromosomes of the Oriental bell-toad, *Bombina orientalis*, with special reference to the multiple ring tetrad. — Journ. of Sci. Hiroshima Univ. Ser. B, 6: 53—70.

159. SAX, K. 1935. Variation in chiasma frequencies in *Secale*, *Vicia* and *Tradescantia*. — Cytologia 6: 289—293.

160. — 1937. Effect of variations in temperature on nuclear and cell division. — Amer. Journ. of Bot. 24: 218—235.

161. SHIGENAGA, M. 1937. An experimental study of the abnormal nuclear and cell division in living cells. — Cytologia, FUJII Jub. Vol.: 464—478.

162. SHIMAMURA, T. 1940. Studies on the effect of the centrifugal force upon nuclear division. — Cytologia 11: 186—216.

163. SPARROW, A. H. and WILSON, G. B. 1941. Relational coiling in the chromo-

some spiralisation cycle of *Trillium erectum* L. and *T. grandiflorum* SALISB. — Genetics 26: 169 (Abstr.) (*).

164. STIEVE, H. 1920. Die Entwicklung der Keimzellen des Grottenolmes (*Proteus anguineus*). I. Die Spermatogenese. — Arch. Micr. Anat. 93. II: 141—313.

165. STOHLER, R. 1928. Cytologische Untersuchungen an den Keimdrüsen der mitteleuropäischen Kröten (*Bufo viridis* LAUR., *B. calamita* LAUR. und *B. vulgaris* LAUR.). — Zeitschr. f. Zellforsch. u. mikr. Anat. 7: 400—475.

166. STONE, L. H. and MATHER, K. 1932. The origin and behaviour of chiasmata. IV. Diploid and triploid *Hyacinthus*. — Cytologia 4: 16—25.

167. STRAUB, J. 1936. Untersuchungen zur Physiologie der Meiosis. II. — Zeitschr. f. Bot. 30: 1—57.

168. STURTEVANT, A. and NOVITSKY, E. 1941. The homologies of chromosome elements in the genus *Drosophila*. — Genetics 26: 517—541.

169. SUOMALAINEN, E. 1940. Beiträge zur Zytologie der parthenogenetischen Insekten. I. Coleoptera. — Ann. Acad. Sci. Fenn. Ser. A. Tome LIV, No. 7

170. SWANSON, C. P. 1941. Major coiling and terminalisation of chiasmata in *Tradescantia*. — Genetics 26: 172—173. (Abstr.) (*).

171. SWINGLE, W. W. 1917. The accessory chromosome in a frog possessing marked hermaphroditic tendencies. — Biol. Bull. 33: 70—79.

172. — 1925. Sex differentiation in the Bullfrog (*Rana Catesbiana*). — Amer. Nat. 59: 154—176.

173. SÄVE-SÖDERBERG, G. 1933. The dermal bones of the head and the lateral line system in *Osteolepis macrolepidotus* A. G. — R. Soc. Sci. Uppsala, Nova Acta. Ser. IV. Vol. 9, No. 2.

174. — 1934. Some points of view concerning the evolution of the vertebrates and the classification of this group. — Ark. f. Zool. Bd. 26 A, No. 17: 1—20.

175. — 1935. On the dermal bones of the head in Labyrinthodont Stegocephalians and primitive Reptilia. — Meddelelser om Grønland, Bd. 98, 3: 5—213.

176. — 1936. On the morphology of triassic Stegocephalians from Spitsbergen, and the interpretation of the endocranium in the Labyrinthodonta. — Kungl. Sv. Vetensk. Akad. Handl. 16: 1—181.

177. TCHOU SU. 1931. Étude cytologique sur l'hybridation chez les Anoures. — Arch. Anat. Micr. 27: 1—105.

178. UPCOTT, M. 1935. The cytology of triploid and tetraploid *Lycopersicum esculentum*. — Journ. of Gen. 31: 1—19.

179. — 1936. The mechanics of mitosis in the pollen tube of *Tulipa*. — Proc. Roy. Soc. London, Ser. B, 121: 207—220.

180. WADA, B. 1933. Mikrodissektion der Chromosomen von *Tradescantia reflexa*. — Cytologia 4: 222—229.

181. — 1937. Mikrurgische Untersuchungen lebender Zellen in der Teilung. V. Die Einwirkung des Ammonia-Dampfes auf die Mitose bei den Staubfadenhaarzellen von *Tradescantia reflexa*. — Cytologia, FUJII Jub. Vol.: 785—795.

182. WALKER, C. E. 1925. The meiotic phase in Triton (*Molge vulgaris*). — Proc. Roy. Soc. London, Ser. B, 98: 29—41.

183. VALLÉN, E. 1942. Beiträge zur Kenntnis der Ontogenie und der vergleichenden Anatomie des Schildkrötenpanzers. — Acta Zoologica 23: 1—123.

184. VANDEL, A. 1937. Chromosome number, polyploidy and sex in the animal kingdom. — Proc. Zool. Soc. London, A, 107: 519—541.

185. WATSON, D. M. S. 1940. The origin of frogs. — Trans. Roy. Soc. Edinb. LX, Part 1: 191—231.

186. WESTERGAARD, M. 1940. Studies on cytology and sex determination in polyploid forms of *Melandrium album*. — Dansk Bot. Ark. 10 (5): 1—131.

187. WHITE, M. J. D. 1934. The influence of temperature on chiasma frequency. — Journ. of Gen. 29: 203—215.

188. — 1936. Chiasma-localisation in *Mecostethus grossus* L. and *Merioptera brachyptera* L. (Orthoptera). — Zeitschr. f. Zellforsch. und mikr. Anat. 24: 128—135.

189. — 1940. The heteropycnosis of sex chromosomes and its interpretation in terms of spiral structure. — Journ. of Gen. 40: 67—82.

190. WICKBOM, T. 1941. The sex chromosomes of Cyprinodontidae and of Teleosts in general, with a list of new chromosome numbers in Cyprinodontidae. — Ark. f. Zoologi, Bd. 33 B, No. 10: 1—6.

191. — 1943. Cytological studies in the family Cyprinodontidae. — Hereditas XXIX: 1—24.

192. WINTREBERT, P. 1922. La voute palatine des Salamandridae. — Biol. Bull. France et Belg. 56: 275—426.

193. WITSCHI, E. 1922. Chromosomen und Geschlecht bei *Rana temporaria*. — Zeitschr. f. ind. Abst.- u. Vererb.-Lehre 27: 253—255.

194. — 1923. Über die genetische Konstitution der Froschzwitter. — Biol. Zbl. 43: 83—96.

195. — 1924. Die Entwicklung der Keimzellen der *Rana temporaria* L. I. Urkeimzellen und Spermatogenese. — Zeitschr. f. Zellen- u. Gewebelehre 1: 523—561.

196. — 1929. Studies on sex differentiation in Amphibians. III. Rudimentary hermaphroditism and Y-chromosome in *Rana temporaria*. — Journ. exp. Zool. 54: 157—223.

197. — 1933. Contribution to the cytology of Amphibian germ cells. I. Chromosomes in the spermatocyte divisions of five north American species of toads. — Cytologia 4: 174—181.

198. WU, J. S. 1938. Chromosome behaviour in three species of grasshoppers. — Cytologia 9: 334—346.

199. YAMAHA, G. 1935. Über die pH-Schwankung in der sich teilenden Pollenmutterzelle einiger Pflanzen. — Cytologia 6: 523—526.

200. — 1938. Über die Ionenkonzentration des Antherenschleimes bei *Lilium*-Arten. — Proc. Imp. Acad. Tokyo, 14: 81—82.

201. ÖSTERGREN, G. 1944. Colchicine mitosis, chromosome contraction, narcosis and protein chain folding. — Hereditas XXX: 429—467.

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DIE KOPPELUNGSGRUPPE N—Z—Fa—Td VON PISUM

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(With a Summary in English)

EINLEITUNG.

DIE Wirkung der vier in der Überschrift genannten Gene *N*, *Z*, *Fa* und *Td* kann folgendermassen charakterisiert werden.

Das Gen *N* beeinflusst sowohl Form, Beschaffenheit wie Länge und Breite der Hülse (vgl. MEUNISSIER, 1918; WELLENSIEK, 1925; E. NILSSON, 1929; LAMPRECHT, 1938). *N* zeigt anscheinend vollkommene Dominanz über *n*. *N*-Hülsen sind je nach ihrer übrigen genotypischen Konstitution: stumpf und gerade = *Bt Cp*, stumpf und gekrümmt = *Bt cp*, spitz und gerade = *bt Cp* sowie spitz und gekrümmt = *bt cp*. *n*-Pflanzen haben stets mehr oder weniger spitze und gekrümmte Hülsen, ganz gleichgültig ob sie in den Genen *Bt* und *Cp* dominant oder rezessiv sind. Das rezessive Gen *n* ist demnach in seiner Wirkung gegenüber der der Genpaare *Bt—bt* und *Cp—cp* epistatisch. Die Stärke der Krümmung von *n*-Hülsen ist jedoch stark abhängig von ihrer Länge; kurze, 4—5 cm lange Hülsen sind fast gerade, 10—12 cm lange Hülsen sind stärker gekrümmt als *N cp*-Hülsen gleicher Länge.

All dies gilt bei gleichzeitiger Dominanz in Gen *Con*. *N con*-Hülsen sind konvex gekrümmt, *n con*-Hülsen sind gerade. Das Gen *con* hebt also, zum Unterschied von *cp*, die durch *n* bedingte Krümmung der Hülsen auf. *N*-Hülsen haben normaldicke, *n*-Hülsen etwa doppelt so dicke Wand. Im Zusammenhang hiermit sind *n*-Hülsen stets voll und schön gerundet, gleichgültig ob es sich um Kneifel- oder Zuckererbsen handelt. Charakteristisch ist ferner für *n*-Hülsen die vom Stiel an viel allmählicher zunehmende Breite als bei *N*-Hülsen, die gleich ihre grösste Breite erreichen. Schliesslich sind *n*-Hülsen stets kürzer als *N*-Hülsen von im übrigen gleicher genotypischer Konstitution. Ganz analog ist bei *n*-Hülsen der Abstand von der Rücken- zur Bauchnaht geringer als bei *N*-Hülsen. Es ist daher unmöglich *n*-Hülsen mit der grossen Breite von gewissen *N*-Hülsen entsprechendem Durchmesser zu erhalten. Schliesslich bedingt die Anwesenheit von Membran (*PV*) in

n-Hülsen eine Verkürzung und damit weniger starke Krümmung und weniger spitzes Ende derselben. Da *n*-Hülsen beim Versuch sie zu biegen gleich Brechbohnen abbrechen, werden *n*-Sorten als Brecherbsen bezeichnet.

Das Gen *Z* ist als ein Grundgen für die Ausbildung der Teilfarbigkeit der Testa aufzufassen (vgl. LAMPRECHT und ÅKERBERG, 1939). Bei *Z*-Pflanzen ist die Testa, sofern diese überhaupt gefärbt ist (Gen *A*), in ihrer Gänze farbig. Bei *A z*-Pflanzen ist die Testa in verschiedener Weise teilfarbig. Für die Ausbildung der verschiedenen Teilfarbigkeit sind bisher die Gene *Mp*, *Dem*, *Cal*, *Lob* und *Pal* festgestellt worden (H. und O. TEDIN, 1928; LAMPRECHT und ÅKERBERG, 1939). Bei gewissen Kombinationen der rezessiven Allelen dieser Gene wird die Testafarbe ganz ausgelöscht, wodurch Samen resultieren, die von solchen der Genkonstitution *aa* nicht unterschieden werden können.

Das durch das Genpaar *Fa—fa* bedingte Eigenschaftspaar, gewöhnlicher runder bzw. *fasciata*-Stamm, war schon MENDEL (1865) bekannt. *Fa* ist vollkommen dominant. *fa*-Pflanzen von *Pisum* scheinen stets mehr oder weniger deutlich fasziierten Stamm zu besitzen; nur bei schwach entwickelten Pflanzen (namentlich bei dichtem Bestand) ist das *fa*-Merkmal mitunter wenig, nur an einem der obersten Internodien, oder gar nicht ausgebildet.

Das Gen *Td* schliesslich bedingt in seiner dominanten Form gezähnte, in seiner rezessiven ganzrandige Blättchen. Eine zahlenmässige Analyse der Spaltung gezähnte : ganzrandige Blättchen scheint bisher nicht veröffentlicht zu sein. SUTTON (1913, S. 359—361) kreuzte eine in Palästina wildwachsende, purpurblütige Erbse mit gezähnten Blättchen mit einer weissblütigen »Speiseerbse» mit ganzrandigen Blättchen. Über F_1 und F_2 wird nur gesagt: »Die Zähnung der Blättchen in modifizierter Form trat bei den F_1-Pflanzen allgemein auf, während sie bei einigen F_2-Pflanzen fehlte». Zahlen für die Aufspaltung werden nicht mitgeteilt. Auch E. v. TSCHERMAK (1919) kreuzte eine Erbsensorte mit gezähnten mit einer mit ganzrandigen Blättchen und erwähnt als Ergebnis, dass die Zähnung der Blättchen dominant ist und monofaktoriell spaltet. Auch v. TSCHERMAK gibt keine Spaltungszahlen an. WELLENSIEK (1925 a) schlug für das Zähnungsgen das Symbol *Td* vor.

Von der zu besprechenden Koppelungsgruppe *N—Z—Fa—Td* ist bisher nur die Koppelung zwischen *Z* und *Fa* bekannt gewesen. WINGE (1936, S. 348) veröffentlichte für diese folgende in zwei Kreuzungen erhaltene Spaltungszahlen:

	702 *Z Fa* :	64 *Z fa* :	72 *z Fa* :	169 *z fa*
D/m für				
9 : 3 : 3 : 1 =	+ 8,61	— 10,06	— 9,45	+ 13,81
	355 *Z Fa* :	151 *Z fa* :	164 *z Fa* :	4 *z fa*
D/m für				
9 : 3 : 3 : 1 =	— 1,88	+ 2,43	+ 3,69	— 6,05

Die erste Kreuzung entsprach dem Typus *Z Fa* × *z fa* und zeigte laut WINGE ein Crossover von 15,1 %, die zweite entsprach dem Typus *Z fa* × *z Fa* und zeigte ein Crossover von 17,7 %. Die erhaltenen Crossoverwerte stimmen gut miteinander überein. Die Koppelung zwischen *Z* und *Fa* kann daher als vollkommen gesichert betrachtet werden.

Auf in der Literatur erwähnte Koppelungen zwischen den hier in Rede stehenden und anderen Genen soll später eingegangen werden. — Vor der Besprechung der Koppelungsuntersuchungen folgt hier zuerst eine Analyse der Vererbung des Eigenschaftspaares gezähnte—ungezähnte Blättchen.

DIE VERERBUNG DER BLÄTTCHENZÄHNUNG.

Laut den in der Einleitung mitgeteilten Angaben von SUTTON (1913) und E. v. TSCHERMAK (1919) ist die Zähnung der Blättchen von *Pisum* eine dominante Eigenschaft. F_1 war stets gezähnt; Spaltungszahlen für F_2 sind noch nicht veröffentlicht.

Die Beurteilung der Zähnung am hier zu besprechenden Material erfolgte in Übereinstimmung mit der in Fig. 1 wiedergegebenen Skala. In bezug auf die F_2-Generation einer Kreuzung zwischen einer ungezähnten und einer gezähnten Linie hat sich nun herausgestellt, dass anscheinend alle Zähnungsgrade von ganz ungezähnt (*td*) bis etwa zum Zähnungsgrad 6 vorkommen. Die gezähnte Elternlinie zeigte einen zwischen 4 und 6 variierenden Zähnungsgrad. Zwecks Klarlegung dieser Verhältnisse wurde der Zähnungsgrad sowohl an einer Reihe von Linien wie in Kreuzungen studiert. Hierbei ergab sich folgendes. *td*-Pflanzen haben entweder ganz ungezähnte Blättchen oder auch solche mit dem Zähnungsgrad 1; vereinzelt trifft man auch Pflanzen mit Zähnung 1—2 oder 1—3. Der Zähnungsgrad 1 bzw. 1—2 und 1—3 ist solchenfalls gewöhnlich an den Blättchen der Stammverzweigungen anzutreffen, während die Blättchen des Hauptstammes ganz ungezähnt sein können. Die Neigung zur Ausbildung des Zähnungsgrades 1 (und vereinzelt 1—3)

ist jedoch bei den einzelnen Linien deutlich verschieden. Für gewisse Linien wurde während den letzten vier Jahren nur *td*, also ganz ungezähnt, vermerkt, andere variierten in der gleichen Zeit von *td* bis 1—3, entsprachen aber in der Hauptsache dem Grad 1.

Von Interesse ist in diesem Zusammenhang das Ergebnis einer Kreuzung (Nr. 534) zwischen Linie 608, die bisher stets ganz ungezähnt war, und Linie 576 mit in der Hauptsache Zähnung 1. Die F_1-Pflanzen

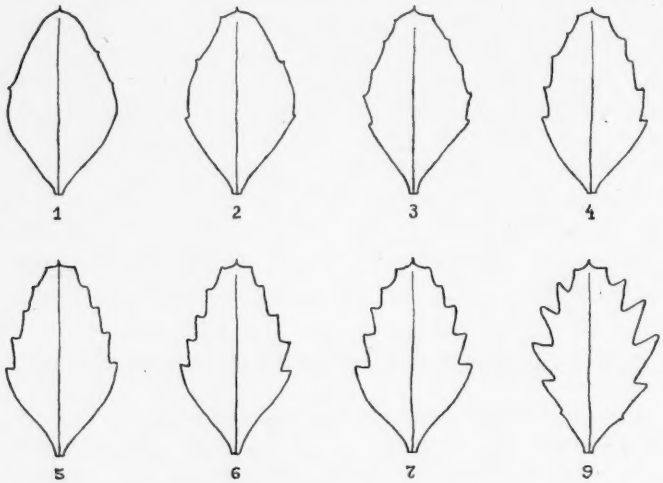

Fig. 1. Die Zähnungsgrade der Blättchen von *Pisum sativum*. (Näheres s. Text.)

waren durchweg ganz ungezähnt. Die F_2-Generation zeigte folgende Zusammensetzung:

Ganz ungezähnt	Mit Zähnung 1 (*td*—1)	Mit Zähnung 1—3
249 = 58,2 %	160 = 37,4 %	19 = 4,4 %

Hierzu ist zu bemerken, dass die Pflanzen mit Zähnung 1 sowie mit 1—3 überdies auch ganz ungezähnte Blättchen tragen konnten. Auffallend war in F_2, dass von den 13 Pflanzen einer Familie, die als schwach und kränklich vermerkt worden ist, 6 die Zähnung 1 und 7 die Zähnung 1—3 zeigten; eine ganz ungezähnte Pflanze gab es also überhaupt nicht. Die dritte Generation dieser Kreuzung bestätigte, dass verschiedene Pflanzen nicht dieselbe Tendenz zur Ausbildung der Zähnung 1 (*td*—1) bzw. 1—3 besitzen. Es ergab sich folgendes:

Nachkommen nach	Ganz ungezähnt	Mit Zähnung 1	Mit Zähnung 1—3
ganz ungezähnten Pflanzen:	50 = 76,9 %	13 = 20,0 %	2 = 3,1 %
Pflanzen mit Zähnung 1: ..	198 = 56,9 »	135 = 38,8 »	15 = 4,3 »

Von den im allgemeinen schwächeren Pflanzen mit Zähnung 1—3 wurden keine Samen gesät. Die Tendenz zur Ausbildung ganz ungezähnter Blättchen ist unter den Nachkommen von F_2-Pflanzen mit solchen Blättchen deutlich grösser als unter solchen mit dem Zähnungsgrad 1.

Die mitgeteilten Ergebnisse scheinen mir aus dreierlei Gründen dafür zu sprechen, dass bei Pflanzen der Konstitution *td td* der Zähnungsgrad 1 und vereinzelt 1—3 hauptsächlich im Zusammenhang mit erblich oder modifikativ bedingter geringerer Vitalität auftritt. Erstens tritt diese schwache Zähnung hauptsächlich auf den Stammverzweigungen auf, die nicht dieselbe volle Wuchskraft haben wie der Hauptstamm. Zweitens wurde diese schwache Zähnung an F_1-Pflanzen, die zufolge Heterosiswirkung fast stets grosse Vitalität aufweisen, nicht beobachtet trotzdem der eine Elter häufig den Zähnungsgrad 1 aufwies. Und drittens zeigten von den Pflanzen einer als schwach und kränklich vermerkten Familie der oben erwähnten Kreuzung Nr. 534 etwa zur Hälfte den Zähnungsgrad 1 und zur Hälfte 1—3, während ganz ungezähnte Individuen überhaupt nicht vorkamen.

Das vorstehend Angeführte spricht dafür, dass die im allgemeinen ungezähnten *td*-Pflanzen — im Zusammenhang mit erblich oder modifikativ bedingter geringerer Vitalität — auch den in Fig. 1 abgebildeten Zähnungsgrad 1 bzw. vereinzelt (1—)3 aufweisen können. Diese schwache Zähnelung kommt solchenfalls vorzugsweise auf den Stammverzweigungen vor. *Td*-Pflanzen von *Pisum sativum* zeigen im allgemeinen den Zähnungsgrad 4—6, mit einer ab und zu vorkommenden Variation bis zu 3 und 7. Auch ein stark gezähnter Typus vom Grad 8—9 kommt vor. Bei diesem sind, wie Fig. 1 zeigt, die Zähne auch spitzer. Die Vererbung dieses Typus, der mit der Zähnung von *P. abyssinicum* übereinzustimmen scheint (s. v. ROSEN, 1944, S. 277), wird hier nicht zum Gegenstand der Untersuchung gemacht. In meinem Kreuzungsmaterial ist dieser Typus bisher zweimal spontan aufgetreten. Wahrscheinlich ist er als Mutation aufzufassen und könnte solchenfalls durch ein drittes Allel des Genpaares *Td—td* bedingt werden.

Es folgen nun die Ergebnisse von drei Kreuzungen, in denen das Genpaar *Td—td* spaltete, und die mit Berücksichtigung des oben Mitgeteilten beurteilt worden sind.

Kreuzung Nr. 229: *L. 20* × *L. 207*. — Linie 20 stammt aus der dänischen Ackererbse Glaenö mit ganz ungezähnten Blättchen. Linie 207 stammt aus der bekannten Kneifelerbse Petit Provençal (= Phänomen, Fenomen) mit gezähnten Blättchen (gewöhnlich Grad 5 laut Fig. 1). Sie ist überdies in einem Gen für Chlorophyll (*xantha*) heterozygot. Die Pflanzen der F_1-Generation zeigten normale Zähnung, die der des einen Elters, Linie 207, entsprach. F_2 gab das folgende Spaltungsverhältnis in bezug auf Zähnung:

514 *Td* : 180 *td* mit D/m für 3 : 1 = + 0,57.

Die Spaltung ist einwandfrei monohybrid mit einem unbedeutenden Überschuss an Rezessiven (6,5 Individuen).

Kreuzung Nr. 233: *L. 58* × *L. 234*. — Linie 58 stammt aus der deutschen rotblütigen Zuckererbse Graue Posthörnchen und hat ungezähnte Blättchen mit einer Variation von *td* bis 1—3. Linie 234 hat normal gezähnte Blättchen (5—6) und ist überdies im Gen *Ast* dominant (Näheres s. LAMPRECHT, 1937). Die F_1-Pflanzen hatten wiederum normal gezähnte Blättchen (4—5—6) und in F_2 wurde folgende Spaltung beobachtet:

352 *Td* : 128 *td* mit D/m für 3 : 1 = + 0,84.

Das Ergebnis entspricht in allen Hinsichten dem in der vorigen Kreuzung Nr. 233 gefundenen.

Kreuzung Nr. 258: *L. 6* × *L. 20*. — Linie 6 repräsentiert den schon von MENDEL erwähnten »*umbellatum*»-Typus mit *fasciata*-Stamm und *lathyrus*-farbigen Blüten (*A Ar b*). Linie 20 s. oben unter Kreuzung 229. Die F_1-Generation zeigte wiederum normale Zähnung (5—6) und die F_2 ergab folgende Spaltung:

347 *Td* : 132 *td* mit D/m für 3 : 1 = + 1,29.

Bei Vereinigung der Spaltungszahlen aller drei Kreuzungen ergibt sich:

1213 *Td* : 440 td mit D/m für 3 : 1 = + 1,52.

Auffallend ist hier, dass in allen drei Kreuzungen ein kleines Plus an Rezessiven vorhanden ist. Wahrscheinlich ist diese Erscheinung dadurch bedingt, dass einige wenige *td*-Pflanzen in jeder Kreuzung mit dem Zähnungsgrad 3 beurteilt und so den *Td*-Pflanzen zugerechnet worden sind. Auf die nun zu besprechenden Koppelungserscheinungen hat dies jedoch keinen Einfluss von Bedeutung.

DIE KOPPELUNGSGRUPPE N—Z—Fa—Td.

Die Berechnung des Crossoverprozentes erfolgte stets nach der bekannten Produktmethode. Eine vorherige Korrektur auf das monohybride 3 : 1-Verhältnis fand in keinem Fall statt, da sich die Werte hierdurch nicht um mehr als höchstens 0,5 % Crossover geändert haben würden, also eine Abweichung, die weit unter der Grösse der mittleren Fehler der Crossoverprozente liegt. Die Berechnung der mittleren Fehler erfolgte mit der von STERN (1933) hierfür umgearbeiteten IMMERschen Tabelle (1930). Für unerlässlich halte ich bei Koppelungsstudien die Angabe der je Kreuzung gesäten Samen und die aus diesen erhaltene Anzahl in jeder Hinsicht beurteilbarer Pflanzen. Nur hierdurch und im Zusammenhang mit der statistischen Sicherheit der erhaltenen Koppelungsverhältnisse kann sich nämlich der Leser ein Urteil über den Wert der veröffentlichten Zahlen bilden. Denn wenn z. B. in einer Kreuzung ein Drittel der F_2-Samen sich nicht zu beurteilbaren Individuen entwickelt, so kann dann keine statistische Methode der Welt mehr klarlegen, welche Genotypen die diesen nichtentwickelten Samen entsprechenden Pflanzen repräsentiert haben würden. — Von einer Angabe der *P*-Werte für die erhaltenen Spaltungsverhältnisse sehe ich ab, da diese die Wahrscheinlichkeit für die Übereinstimmung mit den erwarteten Verhältnissen in viel roherem Grade angeben als die D/m-Werte.

Kreuzung Nr. 60: L. 6 × L. 110. — Linie 6 ist schon oben als *lathyrus*-blütiger *umbellatum*-Typus erwähnt worden. Linie 110 stammt aus der Brecherbse Kungs (aus Roi des Gourmands) und hat, soweit dies hier von Interesse ist, die Genenformel *a m B n Fa v*. Die Formel von L. 6 ist entsprechend *A M b N fa V*. F_1 zeigte die erwartete Dominanz in diesen sechs Genen. Semi- oder partielle Sterilität ist in Kr. 60 nicht aufgetreten. F_2 wurde 1935 mit 500 Samen gesät, die 452 beurteilbare Pflanzen gaben, und 1936 gaben weitere 500 Samen 470 beurteilbare Individuen. Da die Ergebnisse der beiden Jahre nicht voneinander abweichen, werden die Zahlen unten gemeinsam behandelt. An einfachen Genspaltungen wurden erhalten:

735 *Fa* : 187 *fa* mit D/m für 3 : 1 = — 3,31
705 *N* : 217 *n* » » » » = — 1,03
698 *A* : 224 *a* » » » » = — 0,49
690 *M* : 232 *m* » » » » = + 0,11
721 *V* : 201 *v* » » » » = — 2,24
540 *B* : 158 *b* » » » » = — 1,44 (nur *A*-Individuen).

Von diesen monohybriden Spaltungsverhältnissen sind die in den Genen *N*, *A*, *M* und *B* als ungestört zu betrachten. Die beiden Genpaare *V*—*v* und *Fa*—*fa* zeigen dagegen ein deutliches Defizit an Rezessiven. Bei den *v*-Pflanzen ist dieses in der Hauptsache auf die in Kreuzungsmaterial stark erhöhte Mutationsfrequenz von *v* → *V* zurückzuführen (vgl. LAMPRECHT, 1941). In bezug auf *fa*-Pflanzen ist schon in der Einleitung hervorgehoben worden, dass der *fasciata*-Stamm bei schwach entwickelten Individuen mitunter nur an einem einzigen höheren Internodium oder überhaupt nicht zutagetritt. Das Defizit an *fa*-Pflanzen ist sicherlich hierdurch bedingt. Bei den folgenden Zahlenverhältnissen für die Zweigenenspaltungen sind daher die D/m-Werte für Spaltungen, an denen *Fa* bzw. *V* teilnehmen, nicht direkt sondern nach Korrigierung auf Grund der monohybriden Spaltungen berechnet.

Gefunden:	512 *Fa N*	: 210 *Fa n*	: 180 *n Fa*	: 7 *fa n*
Erwartet laut mon. Sp.	562,0 »	: 173,0 »	: 143,0 »	: 44,0 »
D/m für 9 : 3 : 3 : 1 =	— 2,46	+ 3,12	+ 3,12	— 5,03
Gefunden:	550 *Fa A*	: 185 *Fa a*	: 148 *fa A*	: 39 *fa a*
Erwartet:	518,62 »	: 172,87 »	: 172,87 »	: 57,62 »
D/m für 9 : 3 : 3 : 1 =	— 0,43	+ 0,55	+ 0,55	— 0,88
Gefunden:	534 *Fa M*	: 201 *Fa m*	: 156 *fa M*	: 31 *fa m*
D/m für 9 : 3 : 3 : 1 =	— 1,07	+ 1,36	+ 1,36	— 2,19
Gefunden:	581 *Fa V*	: 154 *Fa v*	: 140 *fa V*	: 47 *fa v*
D/m für 9 : 3 : 3 : 1 =	+ 0,41	— 0,52	— 0,52	+ 0,84
Gefunden:	428 *Fa B*	: 122 *Fa b*	: 112 *fa B*	: 36 *fa b*
Erwartet:	392,62 »	: 130,87 »	: 130,87 »	: 43,62 »
D/m für 9 : 3 : 3 : 1 =	+ 0,19	— 0,24	— 0,24	+ 0,39
Gefunden:	530 *N A*	: 175 *N a*	: 168 *n A*	: 49 *n a*
D/m für 9 : 3 : 3 : 1 =	+ 0,76	+ 0,18	— 0,41	— 1,17
Gefunden:	526 *N M*	: 179 *N m*	: 164 *n M*	: 53 *n m*
D/m für 9 : 3 : 3 : 1 =	+ 0,49	+ 0,52	— 0,75	— 0,63

Gefunden:	555 *N V* : 150	*N v* : 166	*n V* : 51	*n v*
D/m für 9 : 3 : 3 : 1 =	+ 0,24	— 0,31	— 0,31	+ 0,50
Gefunden:	410 *N B* : 120	*N b* : 130	*n B* : 38	*n b*
D/m für 9 : 3 : 3 : 1 =	+ 1,33	— 1,05	— 0,08	— 0,83
Gefunden:	534 *A M* : 164	*A m* : 156	*a M* : 68	*a m*
D/m für 9 : 3 : 3 : 1 =	+ 1,02	— 0,75	— 1,42	+ 1,41
Gefunden:	539 *A V* : 159	*A v* : 182	*a V* : 42	*a v*
D/m für 9 : 3 : 3 : 1 =	— 0,45	+ 0,57	+ 0,57	— 0,92
Gefunden:	416 *B M* : 124	*B m* : 118	*b M* : 40	*b m*
D/m für 9 : 3 : 3 : 1 =	+ 1,78	— 0,67	— 1,25	— 0,57
Gefunden:	527 *M V* : 163	*M v* : 194	*m V* : 38	*m v*
D/m für 9 : 3 : 3 : 1 =	— 0,84	+ 1,06	+ 1,06	— 1,71

Für die Gene der obigen Spaltungsverhältnisse ist bisher sichere Koppelung nur zwischen *B* und *M* nachgewiesen. Diese beiden geben allerdings stets einen Crossoverwert von etwa 50 %, aber ihre Koppelung ist durch mehrere im gleichen Chromosom zwischen ihnen gelegene Gene (*F*, *Mp*, *St*, *Gl* u. a.) vollkommen gesichert. Von den gefundenen Zweigenenspaltungen zeigt nur eine Koppelung mit voller Sicherheit an, nämlich die für die beiden Gene *Fa* und *N*. Aus dem gefundenen Spaltungsverhältnis ergibt sich *für die Gene Fa und N ein Crossoverprozent von 20,9 ± 3,12.*

Da WINGE, wie schon einleitend hervorgehoben, zwischen *Z* und *Fa* eine Koppelung mit etwa 16 % Crossover festgestellt hat, war es von Interesse eine Kreuzung zu studieren, in der die drei Gene *Fa*, *N* und *Z* gleichzeitig spalten und an der *N* und *Fa* womöglich in der Koppelungsphase teilnehmen. Meine Kreuzung Nr. 468 erfüllte diese Voraussetzungen.

Kreuzung Nr. 468: L. 490 × L. 560. — Linie 490 stammt aus F_5 der vorstehend beschriebenen Kreuzung 60 und hat, soweit dies hier von Interesse ist, folgende Genenformel: *a b Z Gp Cp V n fa Fl.* Linie 560 stammt aus F_3 von Kreuzung 256. Diese wurde ausgeführt zwischen

L. 20 aus Glaenö (s. o.) und L. 241 aus der deutschen Zuckererbsensorte Goldfähnchen: *a Z Le gp cp v*. Linie 560 hat folgende Genenformel: *A B z gp cp v N Fa fl*. F_1 von Kr. 468 zeigte die laut diesen Formeln erwarteten dominanten Eigenschaften. Semi- oder partielle Sterilität ist in dieser Kreuzung nicht vorgekommen. Zu F_2 wurden 500 Samen gesät, die 451 voll beurteilbare Pflanzen entwickelt haben. An einfachen Genspaltungen wurden erhalten:

368	*Fa*	: 83	*fa*	mit	D/m	für	3 : 1	= — 3,24
350	*N*	: 101	*n*	»	»	»	»	= — 1,28
243	*Z*	: 96	*z*	»	»	»	»	= + 1,41
339	*A*	: 112	*a*	»	»	»	»	= — 0,08
254	*B*	: 85	*b*	»	»	»	»	= + 0,03
325	*Gp*	: 126	*gp*	»	»	»	»	= + 1,44
274	*Cp*	: 76	*cp*	»	»	»	»	= — 1,42
350	*V*	: 101	*v*	»	»	»	»	= — 1,28
337	*Fl*	: 114	*fl*	»	»	»	»	= + 0,14

Von den vorstehenden monohybriden Spaltungsverhältnissen erscheint nur eines, nämlich das im Genpaar *Fa—fa*, gestört. Gleichwie in Kreuzung Nr. 60 besteht hier ein deutliches Defizit an Rezessiven, indem etwa 30 *fa*-Pflanzen zu wenig gefunden wurden. Die Ursache hierfür dürfte stets dieselbe sein: schwächliche Pflanzen bilden keinen *fasciata*-Stamm aus. Die folgenden D/m-Werte sind daher für alle Spaltungen mit *Fa* unter Berücksichtigung der monohybriden Spaltungsverhältnisse korrigiert. Wo dies ausserdem geschah, wurde es durch Hinzufügen von (korr.) angegeben. Für die Spaltung in *Z* und *B* kommen natürlich nur *A*- und für die in *Cp* nur *N*-Individuen in Betracht. Eine eventuelle Beeinflussung der Ergebnisse hierdurch ist wegen der klaren monohybriden Spaltung der Gene *A* und *N* nicht zu befürchten.

Gefunden:	310 *Fa N*	: 58 *Fa n*	: 40 *fa N*	: 43 *fa n*
Erwartet laut mon. Sp.	285,6 »	: 82,4 »	: 64,4 »	: 18,6 »
D/m für 9 : 3 : 3 : 1 =	+ 2,32	— 2,95	— 2,95	+ 4,75
Gefunden:	188 *Fa Z*	: 92 *Fa z*	: 55 *fa Z*	: 4 *fa z*
Erwartet laut mon. Sp.	200,8 »	: 79,2 »	: 42,2 »	: 16,8 »
D/m für 9 : 3 : 3 : 1 =	— 1,40	+ 1,78	+ 1,78	— 2,87

Gefunden:	280 *Fa A*	: 88 *Fa a*	: 59 *fa A*	: 24 *fa a*
Erwartet laut mon. Sp.	276,6 »	: 91,4 »	: 62,4 »	: 20,6 »
D/m für 9 : 3 : 3 : 1 =	+ 0,32	— 0,41	— 0,41	+ 0,66
Gefunden:	212 *Fa B*	: 68 *Fa b*	: 42 *fa B*	: 17 *fa b*
Erwartet laut mon. Sp.	209,8 »	: 70,2 »	: 44,2 »	: 14,8 »
D/m für 9 : 3 : 3 : 1 =	+ 0,25	— 0,33	— 0,33	+ 0,50
Gefunden:	273 *Fa Gp*	: 95 *Fa gp*	: 52 *fa Gp*	: 31 *fa gp*
Erwartet laut mon. Sp.	265,2 »	: 102,8 »	: 59,8 »	: 23,2 »
D/m für 9 : 3 : 3 : 1 =	+ 0,74	— 0,94	— 0,94	+ 1,52
Gefunden:	246 *Fa Cp*	: 64 *Fa cp*	: 28 *fa Cp*	: 12 *fa cp*
Erwartet laut mon. Sp.	242,7 »	: 67,3 »	: 31,3 »	: 8,7 »
D/m für 9 : 3 : 3 : 1 =	+ 0,35	— 0,45	— 0,45	+ 0,73
Gefunden:	290 *Fa V*	: 78 *Fa v*	: 60 *fa V*	: 23 *fa v*
Erwartet laut mon. Sp.	285,5 »	: 82,5 »	: 64,5 »	: 18,5 »
D/m für 9 : 3 : 3 : 1 =	+ 0,43	— 0,54	— 0,54	+ 0,88
Gefunden:	278 *Fa Fl*	: 90 *Fa fl*	: 59 *fa Fl*	: 24 *fa fl*
Erwartet laut mon. Sp.	275,0 »	: 93,0 »	: 62,0 »	: 21,0 »
D/m für 9 : 3 : 3 : 1 =	+ 0,28	— 0,36	— 0,36	+ 0,58
Gefunden:	162 *N Z*	: 94 *N z*	: 81 *n Z*	: 2 *n z*
Erwartet:	190,69 »	: 63,56 »	: 63,56 »	: 21,19 »
D/m für 9 : 3 : 3 : 1 (korr.) =	— 2,35	+ 2,99	+ 2,99	— 4,82
Gefunden:	256 *N A*	: 94 *N a*	: 83 *n A*	: 18 *n a*
Erwartet:	253,69 »	: 84,56 »	: 84,56 »	: 28,19 »
D/m für 9 : 3 : 3 : 1 =	+ 0,22	+ 1,26	— 0,19	— 1,98

Gefunden:	190	*N B* : 66	*N b* : 64	*n B* : 19 *n b*
D/m für				
9 : 3 : 3 : 1 =	— 0,07	+ 0,34	+ 0,06	— 0,49
Gefunden:	259	*N Gp* : 91	*N gp* : 66	*n Gp* : 35 *n gp*
D/m für				
9 : 3 : 3 : 1 (korr.) =	+ 0,65	— 0,82	— 0,82	+ 1,32
Gefunden:	278	*N V* : 72	*N v* : 72	*n V* : 29 *n v*
D/m für				
9 : 3 : 3 : 1 (korr.) =	+ 0,61	— 0,77	— 0,77	+ 1,24
Gefunden:	260	*N Fl* : 90	*N fl* : 77	*n Fl* : 24 *n fl*
D/m für				
9 : 3 : 3 : 1 =	+ 0,60	+ 0,66	— 0,91	— 0,82
Gefunden:	182	*Z B* : 61	*Z b* : 72	*z B* : 24 *z b*
D/m für				
9 : 3 : 3 : 1 =	— 0,95	— 0,36	+ 1,17	+ 0,63
Gefunden:	174	*Z Gp* : 69	*Z gp* : 68	*z Gp* : 28 *z gp*
D/m für				
9 : 3 : 3 : 1 =	— 1,83	+ 0,76	+ 0,63	+ 1,53
Gefunden:	116	*Z Cp* : 46	*Z cp* : 77	*z Cp* : 17 *z cp*
D/m für				
9 : 3 : 3 : 1 (korr.) =	— 0,76	+ 0,96	+ 0,96	— 1,55
Gefunden:	186	*Z V* : 57	*Z v* : 82	*z V* : 14 *z v*
D/m für				
9 : 3 : 3 : 1 (korr.) =	— 0,67	+ 0,85	+ 0,85	— 1,37
Gefunden:	179	*Z Fl* : 64	*Z fl* : 75	*z Fl* : 21 *z fl*
D/m für				
9 : 3 : 3 : 1 =	— 1,28	+ 0,06	+ 1,59	— 0,04
Gefunden:	242	*A Gp* : 97	*A gp* : 83	*a Gp* : 29 *a gp*
D/m für				
9 : 3 : 3 : 1 =	— 1,11	+ 1,50	— 0,19	+ 0,16
Gefunden:	194	*A Cp* : 62	*A cp* : 80	*a Cp* : 14 *a cp*
D/m für				
9 : 3 : 3 : 1 =	— 0,31	— 0,50	+ 1,97	— 1,73
Gefunden:	268	*A V* : 71	*A v* : 82	*a V* : 30 *a v*
D/m für				
9 : 3 : 3 : 1 =	+ 1,36	— 1,65	— 0,31	+ 0,35

Gefunden: 254 *A Fl* : 85 *A fl* : 83 *a Fl* : 29 *a fl*
D/m für
9 : 3 : 3 : 1 = + 0,03 + 0,05 — 0,19 + 0,16

Gefunden: 187 *B Gp* : 67 *B gp* : 55 *b Gp* : 30 *b gp*
D/m für
9 : 3 : 3 : 1 (korr.) = + 0,62 — 0,79 — 0,79 + 1,28

Gefunden: 144 *B Cp* : 46 *B cp* : 49 *b Cp* : 17 *b cp*
D/m für
9 : 3 : 3 : 1 = ± 0,00 — 0,32 + 0,16 + 0,26

Gefunden: 205 *B V* : 49 *B v* : 63 *b V* : 22 *b v*
D/m für
9 : 3 : 3 : 1 (korr.) = + 0,46 — 0,58 — 0,58 + 0,94

Gefunden: 188 *B Fl* : 66 *B fl* : 66 *b Fl* : 19 *b fl*
D/m für
9 : 3 : 3 : 1 = — 0,29 + 0,34 + 0,34 — 0,49

Gefunden: 230 *Gp Cp* : 29 *Gp cp* : 44 *gp Cp* : 47 *gp cp*
D/m für
9 : 3 : 3 : 1 (korr.) = + 2,93 — 3,72 — 3,72 + 5,99

Gefunden: 256 *Gp V* : 69 *Gp v* : 94 *gp V* : 32 *gp v*
D/m für
9 : 3 : 3 : 1 (korr.) = + 0,36 — 0,46 — 0,46 + 0,74

Gefunden: 235 *Gp Fl* : 90 *Gp fl* : 102 *gp Fl* : 24 *gp fl*
D/m für
9 : 3 : 3 : 1 (korr.) = — 0,74 + 0,94 + 0,94 — 1,52

Gefunden: 212 *Cp V* : 62 *Cp v* : 66 *cp V* : 10 *cp v*
D/m für
9 : 3 : 3 : 1 (korr.) = — 0,60 + 0,77 + 0,77 — 1,23

Gefunden: 199 *Cp Fl* : 75 *Cp fl* : 61 *cp Fl* : 15 *cp fl*
D/m für
9 : 3 : 3 : 1 = + 0,23 + 1,28 — 0,63 — 1,52

Gefunden: 281 *V Fl* : 69 *V fl* : 56 *v Fl* : 45 *v fl*
D/m für
9 : 3 : 3 : 1 (korr.) = + 1,85 — 2,35 — 2,35 + 3,79

Von den vorstehend mitgeteilten Zweigenenspaltungen von Kreuzung 468 zeigen fünf sichere Koppelung an. Diese beziehen sich auf die Genpaare *Fa—N*, *Fa—Z*, *N—Z*, *Gp—Cp* und *V—Fl*. Die Spaltungs-

verhältnisse der übrigen 28 Kombinationen von Genen zeigen keine Koppelung an. Diese Genspaltungen sowie die beiden Koppelungsgruppen *Gp—Cp* und *V—Fl* sollen erst später gemeinsam mit entsprechenden Ergebnissen der übrigen Kreuzungen besprochen werden.

Für die Koppelung zwischen den Genen *Fa* und *N* wurde früher in Kreuzung 60 (922 Individuen; Repulsionsphase) ein Crossoverprozent von $20{,}9 \pm 3{,}12$ festgestellt. In die vorliegende Kreuzung 468 sind diese beiden Gene in der Koppelungsphase eingegangen. Eine Berechnung des Crossoverprozentes für *Fa—N* ergibt hier $27{,}8 \pm 2{,}56$. Diese beiden Werte sind mit Hinblick auf die Fehlergrenzen noch gut miteinander zu vereinigen. Gestützt auf die Ergebnisse der beiden Kreuzungen Nr. 60 und 468 dürfte anzunehmen sein, dass *der Crossoverwert für Fa—N bei etwa 25 % liegt.*

Für die Koppelung zwischen *N* und *Z*, die hier zum erstenmal untersucht worden ist, ergibt sich *ein Crossoverwert von* $14{,}8 \pm 5{,}30$ *%*. Für die beiden Gene *Fa* und *Z* hat WINGE (l. c.) in der Koppelungsphase (laut meiner Berechnung) einen Wert von $15{,}0 \pm 1{,}23$ % und in der Repulsionsphase einen solchen von $16{,}5 \pm 3{,}72$ % gefunden. In der vorliegenden Kreuzung Nr. 468 berechnet sich der entsprechende Koppelungswert zu $25{,}0 \pm 5{,}03$ %. Mit Hinblick auf den ziemlich grossen mittleren Fehler der letzteren Bestimmung dürfte auf Grund der bisher vorliegenden Zahlen *für Fa—Z ein Crossoverwert von etwa 18 % anzunehmen sein.*

Die oben besprochenen Ergebnisse berechtigen zu einer sicheren Vereinigung der drei Gene *N*, *Z* und *Fa* in eine Koppelungsgruppe. Die Ordnungsfolge dieser Gene und ihre gegenseitigen Abstände, ausgedrückt durch die Crossoverprozente, entsprechen wahrscheinlich der folgenden:

$$N \text{ —— } 15\ \% \text{ —— } Z \text{ —— } 18\ \% \text{ —— } Fa$$
$$N \text{ ———————— } 25\ \% \text{ ———————— } Fa$$

Kreuzung Nr. 258: *L. 6* × *L. 20.* — Beide diese Linien sind schon früher beschrieben worden. Mit Hinblick auf die genotypische Konstitution ist die Kreuzung zu schreiben: L. 6, *fa Z b D Fl Td* × L. 20, *Fa z B* D^w *fl td.*

F_1 zeigte die erwarteten dominanten Eigenschaften. Die Kreuzung war normal fertil. In F_2 wurden 500 Samen gesät, von denen sich 472 zu voll beurteilbaren Pflanzen entwickelt haben. Die sechs genannten Gene spalteten folgendermassen:

359 *Fa* : 120 *fa* mit D/m für 3 : 1 = + 0,03
348 *Z* : 131 *z* » » » » = + 1,19
346 *B* : 133 *b* » » » » = + 1,40
346 D^w : 133 *D* » » » » = + 1,40
351 *Fl* : 128 *fl* » » » » = + 0,87
347 *Td* : 132 *td* » » » » = + 1,29

Sämtliche obigen monohybriden Spaltungsverhältnisse zeigen gute Übereinstimmung mit dem theoretisch erwarteten Verhältnis. Irgendwelche Störungen scheinen hier demnach nicht vorzukommen. Ungewöhnlich und auffallend erscheint aber, dass alle Verhältnisse ein Plus an Rezessiven zeigen. Gewöhnlich ist das Gegenteil der Fall. Aber in dieser Kreuzung hat sich ein ungewöhnlich hoher Prozent, 95,8 %, aller gesäten F_2-Samen zu voll beurteilbaren Individuen entwickelt. Da dieser Prozent bei Kreuzungen mit dem so häufigen Defizit an Rezessiven 90 meistens nicht übersteigt, spricht dies direkt dafür, dass der Rezessivenmangel in der Regel durch schwache Vitalität von Samen und jungen Pflänzchen im Zusammenhang mit Belastung dieser durch rezessive Gene bedingt wird. — Für die Zweigenenspaltungen wurden folgende Zahlen erhalten.

Gefunden:	233 *Fa Z*	: 126 *Fa z*	: 115 *fa Z*	: 5 *fa z*
Erwartet:	269,44 »	: 89,81 »	: 89,81 »	: 29,94 »
D/m für 9 : 3 : 3 : 1 (korr.) =	— 2,56	+ 3,25	+ 3,25	— 5,25
Gefunden:	263 *Fa B*	: 96 *Fa b*	: 83 *fa B*	: 37 *fa b*
D/m für 9 : 3 : 3 : 1 =	— 0,59	+ 0,72	— 0,79	+ 1,33
Gefunden:	272 *Fa* D^w	: 87 *Fa D*	: 74 *fa* D^w	: 46 *fa D*
D/m für 9 : 3 : 3 : 1 (korr.) =	+ 1,17	— 1,49	— 1,49	+ 2,40
Gefunden:	264 *Fa Fl*	: 95 *Fa fl*	: 87 *fa Fl*	: 33 *fa fl*
D/m für 9 : 3 : 3 : 1 =	— 0,50	+ 0,61	— 0,33	+ 0,58
Gefunden:	238 *Fa Td*	: 121 *Fa td*	: 109 *fa Td*	: 11 *fa td*
D/m für 9 : 3 : 3 : 1 (korr.) =	— 2,03	+ 2,59	+ 2,59	— 4,17

Gefunden: 254 *Z B* : 94 *Z b* : 92 *z B* : 39 *z b*
D/m für
9 : 3 : 3 : 1 (korr.) = + 0,24 — 0,30 — 0,30 + 0,49

Gefunden: 241 *Z D^w* : 107 *Z D* : 105 *z D^w* : 26 *z D*
D/m für
9 : 3 : 3 : 1 (korr.) = — 0,96 + 1,22 + 1,22 — 1,96

Gefunden: 251 *Z Fl* : 97 *Z fl* : 100 *z Fl* : 31 *z fl*
D/m für
9 : 3 : 3 : 1 (korr.) = — 0,37 + 0,47 + 0,47 — 0,76

Gefunden: 271 *Z Td* : 77 *Z td* : 76 *z Td* : 55 *z td*
D/m für
9 : 3 : 3 : 1 (korr.) = + 1,74 — 2,22 — 2,22 + 3,56

Gefunden: 253 *B D^w* : 93 *B D* : 93 *b D^w* : 40 *b D*
D/m für
9 : 3 : 3 : 1 (korr.) = + 0,29 — 0,36 — 0,36 + 0,59

Gefunden: 247 *B Fl* : 99 *B fl* : 104 *b Fl* : 29 *b fl*
D/m für
9 : 3 : 3 : 1 (korr.) = — 0,60 + 0,76 + 0,76 — 1,22

Gefunden: 240 *B Td* : 106 *B td* : 107 *b Td* : 26 *b td*
D/m für
9 : 3 : 3 : 1 (korr.) = — 0,98 + 1,24 + 1,24 — 2,00

Gefunden: 259 *D^w Fl* : 87 *D^w fl* : 92 *D Fl* : 41 *D fl*
D/m für
9 : 3 : 3 : 1 (korr.) = + 0,51 — 0,64 — 0,64 + 1,04

Gefunden: 249 *D^w Td* : 97 *D^w td* : 98 *D Td* : 35 *D td*
D/m für
9 : 3 : 3 : 1 (korr.) = — 0,15 + 0,19 + 0,19 — 0,32

Gefunden: 264 *Fl Td* : 87 *Fl td* : 83 *fl Td* : 45 *fl td*
D/m für
9 : 3 : 3 : 1 (korr.) = + 0,94 — 1,15 — 1,15 + 1,85

Von den obigen Spaltungsverhältnissen können nur drei als sicher Koppelung anzeigend aufgefasst werden, nämlich die für die Genpaare *Fa—Z*, *Fa—Td* und *Z—Td*. Von den übrigen 12 Genkombinationen spricht keine für sichere Koppelung. Nur für das Genpaar *Fa—D* be-

steht eine schwache Andeutung für Koppelung, die aber schon wegen der Lage von *Fa* zwischen den beiden Genen *Z* und *Td* (siehe weiter unten) ausgeschlossen werden kann. Eine Berechnung der Stärke der Koppelung zwischen den drei in Rede stehenden Genen führt zu folgenden Zahlen:

$$\begin{aligned} Z &— Fa \text{ Crossover} = 19{,}0 \pm 4{,}37\ \% \\ Fa &— Td \quad » \quad = 28{,}5 \pm 4{,}14\ » \\ Z &— Td \quad » \quad = 37{,}5 \pm 2{,}92\ » \end{aligned}$$

Auf Grund der in vorliegender Kreuzung erhaltenen Ergebnisse können demnach Ordnungsfolge und Abstände für die Gene *Z*, *Fa* und *Td* folgendermassen zum Ausdruck gebracht werden:

$$\begin{aligned} Z &— 19{,}0\ \% — Fa — 28{,}5\ \% — Td \\ Z &— 37{,}5\ \% — Td \end{aligned}$$

Hiervon war die Koppelung zwischen *Z* und *Fa* auf Grund von WINGE (l. c.) und meinen oben für Kr. 468 mitgeteilten Resultaten bereits zu etwa 18 % Crossover festgestellt worden. Die Übereinstimmung mit den hier gefundenen 19 % ist daher als eine sehr gute zu bezeichnen.

Kreuzung Nr. 539: *L. 462* × *L. 612*. — Linie 462 stammt aus F_3 von Kreuzung Nr. 233 (siehe Abschnitt über Vererbung der Zähnung) und hat stark gezähnte Blättchen sowie *n*-Hülsen. Linie 612 stammt aus einer Erbsensorte, die von Dozent HARRY SMITH, Uppsala, in Ngata in Ost-Tibet, ca. 3.500 m ü. d. M. eingesammelt worden ist. L. 612 hat *N*-Hülsen und ganz ungezähnte Blättchen. Diese Kreuzung war semisteril und gab in F_1 52,1 % Fertilität. Dieses Ergebnis war unter der Voraussetzung, dass die Tibet-Linie hinsichtlich Chromosomenstruktur dem Normaltypus angehört, zu erwarten, da L. 462 im *Le—V*-Chromosom transloziert ist und mit dem Normaltypus Semisterilität gibt. Auf diese Verhältnisse soll hier jedoch nicht näher eingegangen werden. In F_2 wurden 500 Samen gesät, die sich zu nur 436 voll beurteilbaren Individuen entwickelten. Die Gene *N* und *Td* spalteten in folgenden Verhältnissen:

$$\begin{aligned} 335\ N &: 101\ n \text{ mit D/m für } 3:1 = -0{,}89 \\ 322\ Td &: 114\ td \quad » \quad » \quad » \quad » = +0{,}55 \end{aligned}$$

Als bifaktorielles Verhältnis ergab sich für diese beiden Gene:

Gefunden:	234 *N Td* :	101 *N td* :	88 *n Td* :	13 *n td*
Erwartet laut mon. Sp.	247,4 » :	87,6 » :	74,6 » :	26,4 »
D/m für 9 : 3 : 3 : 1 (korr.) =	— 1,30	+ 1,65	+ 1,65	— 2,66

Das gefundene bifaktorielle Spaltungsverhältnis zeigt deutliche Koppelung an und eine Berechnung ergibt für die beiden *Gene N und Td einen Crossoverwert von 35,2 ± 3,94 %*.

Eine Zusammenstellung der bisher bekannten Crossoverwerte für die vier zu einer Koppelungsgruppe gehörigen Gene *N*, *Z*, *Fa* und *Td* ergibt folgendes.

N —*Z*	Crossoverwert	laut	Kreuzung	468: 14,8 ± 5,30 %
N —*Fa*	»	»	»	60: 20,9 ± 3,12 »
	»	»	»	468: 27,8 ± 2,56 »
N —*Td*	»	»	»	539: 35,2 ± 3,94 »
Z —*Fa*	»	»	WINGE (1936):	15,0 ± 1,23 »
			und	16,5 ± 3,72 »
	»	»	Kreuzung	258: 19,0 ± 4,37 »
	»	»	»	468: 25,0 ± 5,03 »
Z —*Td*	»	»	»	258: 37,5 ± 2,92 »
Fa—*Td*	»	»	»	258: 28,5 ± 4,14 »

Für zwei der Genkombinationen, nämlich für *N*—*Fa* und *Z*—*Fa*, liegen zwei bzw. vier Werte vor, die jedoch — mit Hinblick auf die Grösse der mittleren Fehler — gut miteinander vereinbar sind. Für *N*—*Fa* erhält man ungefähr 25 % und für *Z*—*Fa* etwa 18 % Crossover. Für die am weitesten auseinander gelegenen Gene *N* und *Z* einerseits sowie *Td* andererseits gehen die Crossoverwerte mit 2 % in die nicht erwartete Richtung. Dies besagt jedoch nichts, da der mittlere Fehler für diese beiden Crossoverwerte etwa 3,5 % beträgt. Die oben vorgelegten Kreuzungsergebnisse (einschliesslich WINGEs Werte für *Z*—*Fa*) berechtigen dazu, die vier zu einer Koppelungsgruppe gehörigen Gene *N*, *Z*, *Fa* und *Td* hinsichtlich Ordnungsfolge und Abstände folgendermassen darzustellen:

N —— 15 % —— *Z* —— 18 % —— *Fa* ——— 28 % ——— *Td*

N ——————— 25 % ——————— *Fa*

BEZIEHUNGEN DER KOPPELUNGSGRUPPE N—Z—Fa—Td ZU ANDEREN GENEN BZW. KOPPELUNGSGRUPPEN.

Im folgenden werden teils in der Literatur vorhandene Angaben über Koppelung von Genen oder Gengruppen mit *N*, *Z*, *Fa* bzw. *Td* kritisch überprüft, teils weitere eigene Kreuzungsresultate zur Beleuchtung dieser Verhältnisse mitgeteilt.

WELLENSIEK (1925) berichtet über Koppelung zwischen den Genen *Bt*, *Cp*, *N*, P_1 (= *Pur*) und *V* (in bezug auf das Symbol *Pur*, durch das P_1 zu ersetzen ist, siehe LAMPRECHT, 1938, S. 151). Die angeführten Crossoverwerte mit den mittleren Fehlern sind von mir berechnet. WELLENSIEKs Kreuzung 12, *Pur V N Cp* × *pur v n cp*, gab folgende Spaltungen:

212	*Pur V*	: 45	*Pur v*	: 40	*pur V*	: 14	*pur v*;	Co = 43,0 ± 3,91	%
191	*N Pur*	: 35	*N pur*	: 66	*n Pur*	: 19	*n pur*;	Co = 43,7 ± 3,93	»
217	*Cp Pur*	: 40	*Cp pur*	: 28	*cp Pur*	: 26	*cp pur*;	Co = 29,2 ± 3,16	»
279	*Cp V*	: 49	*Cp v*	: 53	*cp V*	: 36	*cp v*;	Co = 32,2 ± 2,89	»
269	*N Cp*	: 35	*N cp*	: 59	*n Cp*	: 54	*n cp*;	Co = 25,7 ± 2,51	»

Aus diesen Kreuzungsergebnissen zieht WELLENSIEK den Schluss, dass Koppelung zwischen den Genpaaren *Pur—Cp*, *V—Cp* und *Cp—N* offenbar ist, während die Beziehungen *Pur—V* und *Pur—N* nicht ganz klar sind. Aber mit Hinblick auf MORGANS Theorie, heisst es weiter, müssen auch diese zur selben Koppelungsgruppe gehören. Im Anschluss an die graphische Darstellung der Koppelungsgruppe sagt WELLENSIEK indessen, dass die Beziehungen von *N* zu den anderen Genen dunkel sind, weshalb die ganze Theorie angezweifelt werden kann. In einer weiteren Kreuzung (l. c. Nr. 14) wird für *N—Cp* noch folgendes Verhältnis mitgeteilt:

$$145\ N\ Cp : 27\ N\ cp : 36\ n\ Cp : 29\ n\ cp;\ \text{Co} = 31{,}0 \pm 3{,}74\ \%.$$

Laut WELLENSIEK spricht dies für klare Koppelung.

In einer späteren Arbeit (1928) werden die obigen Koppelungen in Rückkreuzungen geprüft. Nun findet WELLENSIEK nur für die beiden Gene *Cp* (jetzt S_s genannt; Symbolik s. LAMPRECHT, 1942) und *N* eine anscheinend klare Koppelung mit 13,3 bzw. 10,7 % Crossover. Die übrigen Koppelungen konnten nicht bestätigt werden. Aber die Koppelung von *Cp* mit *V*, die etwa 43 % Crossover gab, wird trotzdem als »klar» angeführt. In einer kurz darauf (1929) erschienenen Arbeit über das Auftreten von mehr als 50 % Crossover berichtet WELLENSIEK

über zwei Rückkreuzungen, die wieder für die Gene *N*—*Cp* eine Koppelung von 15,9 bzw 8,9 % Co. anzeigen.

Erwähnt sei noch, dass WELLENSIEK (1925, 1928) auch Koppelung zwischen *Gp* und *Cp* sowie *Bt* und *Cp* feststellen konnte. *Gp* wird daher auch in die Koppelungsgruppe mit *N* einbezogen, obgleich die Spaltungszahlen für das Genpaar *Gp*—*N* unabhängige Vererbung anzeigen und auch kein dazwischenliegendes, vermittelndes Gen hierfür gefunden worden ist. Auf die Koppelung von *Bt* mit *Cp* soll hier nicht eingegangen werden.

Überblicken wir nun die vorstehend angeführten, von WELLENSIEK gefundenen Koppelungen, so wird unmittelbar klar, dass sich alles um das Gen *Cp* dreht. Es werden immer nur Koppelungen mit *Cp* gefunden, während für keines der übrigen Genpaare auch nur eine einigermassen sichere Koppelung festgestellt werden konnte. Und am stärksten und sichersten erscheint die Koppelung zwischen *Cp* und *N*.

Im vorigen Kapitel wurde von mir die Koppelungsgruppe *N*—*Z*—*Fa*—*Td* nachgewiesen und hierbei auch die Beziehung dieser Koppelungsgruppe zu den Genen *A*, *M*, *B*, *V*, *Gp*, *Cp*, *D* und *Fl* untersucht. Alle diese letztgenannten 8 Gene spalteten zweifellos unabhängig von den vier Genen der in Frage stehenden Koppelungsgruppe. Da diese laut den gefundenen Genabständen den grösseren Teil eines Chromosoms einnehmen müssen, dürfte jede Möglichkeit zurückzuweisen sein, dass auch nur noch eines der genannten 8 Gene dieser Koppelungsgruppe angehören kann. Wie ersichtlich gehören hierzu auch die drei Gene *V*, *Gp* und *Cp*, für die WELLENSIEK Koppelung mit *N* findet. Von besonderer Bedeutung ist natürlich, dass *Cp* nicht mit *N* gekoppelt ist, was durch die untersuchten Genkombinationen *Fa*—*Cp* und *Z*—*Cp* sowie durch die klar monohybride Spaltung nach 3 *Cp* : 1 *cp* der *N*-Pflanzen einwandfrei bewiesen erscheint; *n*-Pflanzen können bekanntlich nicht hinsichtlich *Cp*—*cp* beurteilt werden.

Die Beurteilung des Genpaares Cp—cp. — Wie sind nun WELLENSIEKS Ergebnisse zu erklären? In bezug auf das Gen *Cp* ist folgendes anzuführen. Sowohl WELLENSIEK selbst wie auch andere Forscher haben die Schwierigkeiten bei der Klassifikation von *Cp*—*cp* betont. Es ist richtig, dass *Cp* nicht vollkommen dominant ist, aber die *Cpcp*-Hülsen stehen den *CpCp*-Hülsen doch so nahe, dass die Ursache der Schwierigkeiten nicht hierin zu suchen ist. Die Schwierigkeiten werden vielmehr in der Hauptsache durch die übrige genotypische Konstitution des *Cp*—*cp*-Materials bedingt. So ist die Stärke der Krümmung von *cp*-Hülsen in hohem Grad vom Verhältnis Länge/Breite der Hülsen

abhängig. Eine kurze und breite Hülse bekommt niemals die starke Krümmung einer langen und schmalen Hülse. Auch die An- bzw. Abwesenheit der Membran beeinflusst die Krümmung der Hülse. So sind breithülsige (und flache) *cp*-Zuckererbsen (*N Con*) im allgemeinen weniger gekrümmt und schwieriger zu beurteilen als *cp*-Kneifelerbsen. Wenn also eine Kreuzung ausser in *Cp* : *cp* auch in Genen für die Grössenverhältnisse der Hülsen spaltet, was natürlich häufig vorkommt, so verlangt eine solche Kreuzung eine Beurteilung, die diese Verhältnisse besonders berücksichtigt. Geschieht dies nicht und werden nur die ausgeprägt gekrümmten Hülsen als *cp* klassifiziert, so bekommt man dann stets ein mehr oder weniger grosses Defizit an *cp*-Hülsen.

Die Klassifikation von Cp : cp und N : n. — Mit Hinblick auf in diesen Genen spaltenden Kreuzungen ist zu sagen, dass die gleichzeitige Spaltung in diesen beiden Genen überhaupt nicht beurteilt werden kann. Man kann sehr leicht die *N*- von den *n*-Pflanzen unterscheiden. Aber in der *n*-Gruppe kann auf Grund der Phänotypen nicht geschlossen werden, ob eine Pflanze *Cp* oder *cp* ist (vgl. die Charakteristik der Wirkung von *n* in der Einleitung). *n*-Hülsen sind stets mehr oder weniger gekrümmt; kürzere weniger als längere und ganz kurze Hülsen sind fast gerade. Aber dies hat nichts mit dem Genpaar *Cp—cp* zu tun. Man kann natürlich, wenn man so wünscht, die *n*-Hülsen in schwach gekrümmte bis fast gerade und in stark gekrümmte sortieren. Das Ergebnis ist dann (wenn nicht gerade alle Hülsen sehr kurz sind) gewöhnlich, dass man keine 3 : 1-Spaltung sondern zuviel gekrümmte Hülsen findet. Jede dieser Gruppen wird aber dann sowohl *Cp*- wie *cp*-Hülsen enthalten. Eine Klassifikation von *n*-Hülsen in bezug auf *Cp—cp* ist eben, wie schon erwähnt, okular undurchführbar.

Wenn nun die vorstehend besprochenen Verhältnisse bei der Beurteilung der phänotypischen Manifestation der beiden Genpaare *Cp—cp* und *N—n* nicht berücksichtigt werden, so sind gerade die von WELLENSIEK gefundenen Ergebnisse zu erwarten. In der *N*-Gruppe werden dann zuviel *Cp*-Pflanzen und in der *n*-Gruppe zuviel *cp*-Pflanzen gefunden. Und als Ergebnis dieses Beurteilungsüberschusses an *N Cp*- bzw. *n cp*-Pflanzen erhält man die anscheinend gesicherte Koppelung zwischen *N* und *Cp*. Damit glaube ich die Ursachen für die von WELLENSIEK gefundene Koppelung zwischen *Cp* und *N* in befriedigender Weise klargelegt zu haben. Dass diese Koppelung und die indirekt auf Grund dieser angenommenen nicht den tatsächlichen Verhältnissen entsprechen, ist schon durch die von mir oben mitgeteilten Kreuzungsresultate (Kr. 468 u. a.) bewiesen worden.

Das Gen Q für Samenabort. — 1929 beschrieben WELLENSIEK und KEIJSER ein Gen *Q*, das in dominanter Form in mehr oder weniger hohem Grad die Nichtentwicklung von Samenanlagen zu Samen bedingen soll. Einleitend wird l. c. gesagt, dass diese Erscheinung ausser in F_2 auch in reinen Linien und in F_1 angetroffen worden ist. WELLENSIEK und KEIJSER finden insgesamt eine Spaltung nach 891 *Q* : 308 *q* mit D/m für $3 : 1 = + 0{,}55$. Ferner wird festgestellt, dass *N*, *Cp* und *Q* offenbar zu einer Koppelungsgruppe gehören. Als Crossoverwerte werden angeführt: Für *N*—*Q* 16,8 %, für *Cp*—*Q* 14,1 % und für *Cp*—*N* 8,8. %

Wer mit semisterilen Kreuzungen gearbeitet hat und Fig. 1 von WELLENSIEK und KEIJSER betrachtet, wird unmittelbar den Verdacht hegen, dass es sich hier höchst wahrscheinlich um Semi- oder partielle Sterilität, bedingt durch Spaltung in strukturell verschiedenen Chromosomentypen gehandelt hat und nicht um einen Samenausfall, bedingt durch die Wirkung eines dominanten Gens *Q*. Auf Grund der Untersuchungen von PELLEW (1937) und LAMPRECHT (1939) wissen wir nunmehr, dass je nach der Beschaffenheit der Translokationen, die die Ursache der Sterilität bilden, 25-, 33-, 50-, 66- und 75-%ige Fertilität auftreten kann. Auf Grund der von WELLENSIEK und KEIJSER veröffentlichten F_2-Daten kann natürlich nicht entschieden werden, ob es sich wirklich um ein dominantes Gen *Q* handelt oder nicht. Ihre Äusserung (l. c. S. 331) »Die Auslese eines einzigen Jahres ist indessen schon vollkommen effektiv um das Material von der Sterilität zu befreien» ist indessen ebenso gut mit der Annahme vereinbar, dass die hier in Frage stehende partielle Sterilität durch Kreuzung von strukturell verschiedenen Linien bedingt wird. Eine Überprüfung dieser Spaltung in sterile und fertile Individuen erscheint mir daher als eine unabweisbare Forderung für die Anerkennung des Gens *Q*. Bis dies geschehen ist, will ich daher das Bestehen eines solchen Gens *Q* sowie auch seine Aufnahme in eine Koppelungsgruppe als nicht berechtigt betrachten.

DAS SCHICKSAL DER WELLENSIEKSCHEN KOPPELUNGEN ZWISCHEN Cp, N, Q UND ANDEREN GENEN IN DER JÜNGEREN GENETISCHEN LITERATUR.

RASMUSSON (1927, Tab. 30) nimmt in seine Übersicht über Koppelungen bei *Pisum* u. a. folgende Gene als miteinander gekoppelt auf: P_1 (= *Pur*), *Cp*, *B*, *N*, *Btb*, *Gp* und *Bt*. WELLENSIEK und KEIJSERs Gen

Q war damals noch nicht veröffentlicht. Wie ersichtlich sind dies die oben besprochenen Koppelungen von WELLENSIEK. Die Koppelungen P_1—*N*, *B*—*Cp* und *B*—*N* werden von RASMUSSON als unsicher bezeichnet. Es handelt sich hier um jene Koppelungen, die WELLENSIEK selbst bei seinen Rückkreuzungen (1928) nicht mehr bestätigen konnte. Die übrigen Koppelungen P_1—*Cp*, *Gp*—*Cp*, *Bt*—*Cp*, *N*—*Cp* und *Btb*—*Cp* werden von RASMUSSON als sicher bezeichnet, aber man weiss, wird hinzugefügt, dass es von dem einen oder beiden Genen dieser Kombinationen multiple oder komplementäre Gene gibt. Dies kann aber in keinem der Fälle als bewiesen betrachtet werden. Das Gen *Bt*, stumpfe—spitze Hülse, wurde von RASMUSSON selbst (l. c.) in *Bta* und *Btb* auf-

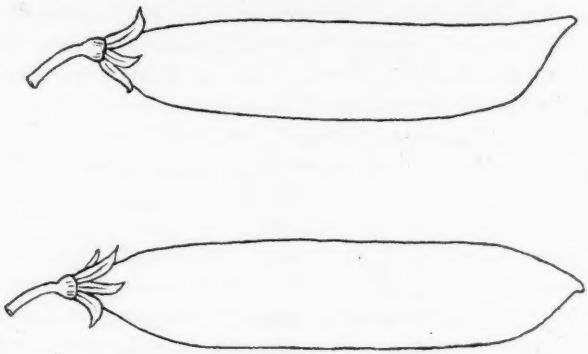

Fig. 2. Einseitig und zweiseitig zugespitzte Zuckererbsenhülse; obere deutlich stumpf, *Bt*, untere unsicher ob stumpf oder spitz.

geteilt (seine Tab. 30 nimmt sowohl *Bt* wie *Bta* und *Btb* auf!), da er in zwei Kreuzungen eine 9 : 7-ähnliche Spaltung gefunden hat. Die Werte für D/m betrugen 2,80 bzw. 4,17. Wie ich schon früher (LAMPRECHT, 1936) hervorgehoben habe, handelte es sich in diesen Fällen um Kreuzungen zwischen Zucker- und Markerbsen, wobei sog. zweiseitig zugespitzte Hülsen von Zuckererbsen (siehe Fig. 2) auftreten, bei denen phänotypisch nicht sicher oder sehr schwer entschieden werden kann, ob sie *bt* oder *Bt* sind. Ihr Auftreten und ihre Klassifikation als *bt* könnte die Ursache der 9 : 7-ähnlichen Spaltungen sein. Entscheidend für das Bestehen von zwei *Bt*-Genen wäre hier eine Kreuzung zwischen zwei spitzhülsigen, die in F_1 Pflanzen mit stumpfen Hülsen geben sollten. Auch liegen keine sicheren Koppelungsverhältnisse für die Begründung von zwei solchen Genen vor. Bindende Beweise für das Bestehen von *Bta* und *Btb* fehlen demnach noch. Auch in bezug auf

Cp scheint RASMUSSON (mit Hinblick auf seine Äusserung über das Vorkommen von multiplen Genen) mit zwei Genen, hier aber von multipler Wirkung, zu rechnen, da er für die Spaltung gerade : gekrümmte Hülse 15 : 1-ähnliche Verhältnisse findet (l. c. Tab. 21 und 25). Auch hier steht der Beweis für die Existenz von zwei *Cp*-Genen aus, die durch Kreuzung zweier geradhülsiger mit Ausspaltung in F_2 von $^1/_{16}$ mit gekrümmten Hülsen zu erbringen wäre. Meiner Ansicht nach ist bei RASMUSSONS Spaltungen in *Cp* : *cp* gleichfalls mit den oben besprochenen Klassifikationsschwierigkeiten zu rechnen.

WINGE nimmt in seine Tab. 6 (1936, S. 363) folgende Koppelungsgruppe auf: *N—Cp—Q—Pa—Btb*. Die Gruppe *N—Cp—Q—Bt* wurde offenbar von WELLENSIEK übernommen. *Bt* wurde laut RASMUSSON (1927) in *Btb* umgeändert und *Pa* wurde auf Grund einer brieflichen Mitteilung von DE WINTON (WINGE, l. c. S. 304) aufgenommen, die für *Pa—Cp* einen Crossoverwert von 22 % gefunden haben soll. In seinem Kommentar zur Koppelungsgruppe 3, wie diese bezeichnet wird, hebt er jedoch hervor, dass eine Kontrolle der Koppelungen *Pa—Btb*, *Q—Pa* und *N—Pa* sowie auch eine allgemeine Revision der Gene dieser Gruppe wünschenswert sei.

Schliesslich erwähnt G. v. ROSEN (1944, S. 275) eine »klassische Koppelungsgruppe» *tl—r—pa—q—cp* und im Kapitel »Genkarten» derselben Arbeit (S. 311) bringt er in seinem Chromosom III folgende Gene unter: *a xa tl r pa q* cp_1 *n* und bt_b. In der »klassischen» Gruppe fehlt das wichtige Gen *n* und auch *btb*. In der Genkarte befinden sich diese jedoch in der Gruppe, der hier überdies *a—xa* angehängt wird. Das Gen X_a, von RASMUSSON (1935) so benannt, ist jedoch synonym mit HOSHINOS (1915) *Lf*. Beide sind mit *A* ziemlich stark gekoppelt, und bevor klar bewiesen ist, dass es zwei solche Gene gibt, hat das ältere Symbol *Lf* zu gelten.

In der Erläuterung zu dieser Koppelungsgruppe sagt v. ROSEN: »Auf Grund der Angaben von NILSSON (1939) und der Ergebnisse vorliegender Untersuchung wurde als berechtigt erachtet, dass RASMUSSONS (1935) kleine Gruppe *a—xa* mit der klassischen Gruppe *tl—r* zu vereinigen ist, welch letztere NILSSON schon mit seiner Gruppe *pa—n—*cp_1*—q—*bt_b vereinigt hat». v. ROSENS Bezugnahme auf E. NILSSON (1939) ist unrichtig. NILSSONS in Rede stehende Untersuchung hat sich nur mit den Genen *Pa*, *R*, *Tl* und *Bt* experimentell beschäftigt. NILSSON schliesst u. a. mit den Worten (l. c. S. 57): »Die nun beendigten Untersuchungen beweisen nur, dass *Pa—R—Tl—Btb* eine Koppelungsgruppe bilden, und dass *Btb* in grossem Abstand von den übrigen Genen liegt».

Von einer Vereinigung irgendwelcher anderer Gene mit dieser Gruppe ist in NILSSONs Arbeit nicht die Rede. Die Aufnahme der Gene *N*, *Cp* und *Q* durch v. ROSEN in diese Gruppe erfolgte wohl nur kompilatorisch, d. h. mit Hinblick auf WELLENSIEKs Ergebnisse sowie zufolge der Übernahme dieser WELLENSIEKschen Koppelungsgruppe in die Arbeiten von RASMUSSON (1927) und von WINGE (1936). In v. ROSENs eigener Arbeit findet man keine Ergebnisse die hierzu berechtigen.

Die Anhängung von A—Lf an die Koppelungsgruppe Pa—R—Tl—Bt durch v. ROSEN (*1944, S. 311*). — Auch diesbezüglich hat NILSSON (1939) keinerlei Kreuzungsergebnisse veröffentlicht, auf die sich v. ROSEN zu berufen können glaubt. Wohl aber hat PELLEW (1937) bei der Kreuzung von strukturellen *Pisum*-Hybriden vom (chromosomalen) Typus 2, 3 und 6 mit dem Normaltypus gefunden, dass wenn der F_1-Bastard gleichzeitig in *R* und *A* heterozygot war, so zeigte F_2 entweder Koppelung zwischen *R* und *A* mit etwa 30 % Crossover oder auch Koppelung beider dieser Gene mit der gametischen Sterilität. Dies scheint tatsächlich dafür zu sprechen, dass *A* und *R* im gleichen Chromosom liegen. Trotzdem kann dies meiner Ansicht nach ohne weitere genetische Überprüfung in Kreuzungen zwischen Linien vom Normaltypus nicht als festgestellt betrachtet werden. Denn es gibt sehr gesicherte Kreuzungsresultate, mit denen diese Annahme unvereinbar erscheint. Es sind dies die Beziehungen zwischen den drei Genen *A*, *R* und *Bt*.

Zwischen den Genen *A* und *R* sowie zwischen *A* und *Bt* findet man stets etwa 50 % Crossover, d. h. unabhängige Vererbung. Einschliesslich meiner eigenen Kreuzungsergebnisse ist die Spaltung in *A*—*a* und *R*—*r* bisher an etwas mehr als 28.000 Individuen mit diesem Resultat untersucht worden. Von meinem eigenen Material erwähne ich hier nur eine 1931 in grossem Umfang analysierte Kreuzung (Nr. 4) zwischen L. 58, aus Graue Posthörnchen, *A R*, und L. 102, aus Acacia, *a r*. Folgende Spaltung wurde erhalten (gesät 5500 Samen; entwickelte Pflanzen 5239):

Gefunden:	2972	*A R* : 989	*A r* : 957	*a R* : 321	*a r*
D/m für 9 : 3 : 3 : 1 =		+ 0,70	+ 0,24	— 0,90	— 0,32

Hierfür ergibt sich ein Crossoverwert von $49{,}8 \pm 1{,}04$ %. SVERDRUPS (1927) Kreuzung mit 4166 Individuen gibt ein Crossover von $49{,}6 \pm 1{,}16$ %. Diese Werte für das Crossover zwischen *A* und *R* liegen so nahe 50 %,

wie man dies nur wünschen kann, und zeigen demnach nicht im geringsten Koppelung an.

Gleiches wie für *A—R* wurde auch für *A—Bt* gefunden. WELLENSIEKS (1925) Ergebnisse einer Kreuzung mit 840 Individuen ergaben einen Crossoverwert von $47{,}7 \pm 2{,}51$ %. KAPPERTS (1924) Kreuzung mit 1352 Individuen gibt $51{,}2 \pm 2{,}02$ % und RASMUSSONS (1927) Kreuzung mit 1012 Individuen $49{,}8 \pm 2{,}36$ % Crossover. Sechs von mir selbst auf diese Genkombination hin untersuchte Kreuzungen mit zusammen 2110 Individuen, erhalten aus 2410 Samen, haben folgende Spaltung gegeben.

Gefunden:	1228 *A Bt*	: 370 *A bt*	: 376 *a Bt*	: 136 *a bt*
D/m für				
9 : 3 : 3 : 1 =	+ 1,81	— 1,43	— 1,09	+ 0,37

Crossoverwert $= 47{,}5 \pm 1{,}60$.

Für die bereits oben erwähnte Kreuzung Nr. 4 mit 3986 *N*-Individuen (Repulsionsphase) ergibt sich:

Gefunden:	2183 *A Bt*	: 835 *A bt*	: 735 *a Bt*	: 233 *a bt*
D/m für				
9 : 3 : 3 : 1 =	— 1,56	+ 3,52	— 0,50	— 0,86

Da hier von den insgesamt 5239 Individuen nur die *N*-Pflanzen (D/m für $3\,N : 1\,n = -1{,}75$) zu dieser Analyse herangezogen werden konnten, ist die Sicherheit des Crossoverwertes geringer als durch den mittleren Fehler angegeben wird. Hierzu kommt noch eine gewisse Klassifikationsschwierigkeit bei der Beurteilung von Zuckererbsen auf *Bt—bt*. Crossoverwert $= 47{,}4 \pm 1{,}22$ %. Die Spaltungsergebnisse für die Genkombination *A—Bt* zeigen, gleichwie die für *A—R*, keine Abweichung von unabhängiger Vererbung an.

Verbleibt schliesslich die Genkombination *R—Bt*. Für diese hat KAPPERT (1924) in einer Kreuzung mit 1354 Individuen folgende Spaltung erhalten:

Gefunden:	812 *R Bt*	: 212 *R bt*	: 179 *r Bt*	: 151 *r bt*
D/m für				
9 : 3 : 3 : 1 =	+ 2,77	— 2,91	— 5,21	+ 7,45

Hierfür berechnet sich ein Crossoverwert von $34{,}3 \pm 1{,}65$ %.

RASMUSSON (1927, Tab. 25) veröffentlicht zwei Kreuzungen, in denen *R* und *Bt* spalten. Beide wurden zwischen Mark- und Zuckererbsen in Koppelungsphase ausgeführt. Folgende Zahlen wurden erhalten:

Gefunden: 619 *R Bt* : 191 *R bt* : 162 *r Bt* : 40 *r bt*
D/m für
9 : 3 : 3 : 1 = + 3,12 + 0,10 — 2,23 — 2,95

Crossoverwert = 52,7 ± 2,23. Wie ersichtlich sind die Zahlen recht unregelmässig, was — wie schon früher besprochen — wahrscheinlich mit Klassifikationsschwierigkeiten bei den Zuckererbsen zusammenhängen dürfte.

Gefunden: 441 *R Bt* : 140 *R bt* : 149 *r Bt* : 45 *r bt*
D/m für
9 : 3 : 3 : 1 = — 0,07 — 0,67 + 0,13 + 1,01
Crossoverwert = 47,7 ± 2,60.

E. NILSSON (1939, S. 54) veröffentlicht ein wertvolles Material für die Sicherung der Beziehung zwischen *R* und *Bt*, nämlich 9 Kreuzungen in Koppelungs- und 4 in Repulsionsphase, die sämtlich zwischen Kneifelerbsen ausgeführt worden sind. Die Klassifikationsschwierigkeiten bei Zuckererbsen fallen hier demnach weg. Für die 9 Kreuzungen vom Typus *R Bt* × *r bt* wurde erhalten:

Gefunden: 1742 *R Bt* : 453 *R bt* : 407 *r Bt* : 288 *r bt*
D/m für
9 : 3 : 3 : 1 = + 4,37 — 4,19 — 6,43 + 8,34
Crossoverwert = 36,5 ± 1,17 %.

Die 4 Kreuzungen vom Typus *R bt* × *r Bt* gaben folgende Spaltung.

Gefunden: 655 *R Bt* : 264 *R bt* : 279 *r Bt* : 45 *r bt*
Crossoverwert = 37,2 ± 1,58 %.

Selbst habe ich die Spaltung in den Genen *R* und *Bt* bisher in 6 Kreuzungen mit folgenden Ergebnissen untersucht.

Kreuzung Nr. 4; L. 58 × L. 102 (s. oben). 3986 *N*-Individuen, Repulsionsphase.

Gefunden: 2121 *R Bt* : 826 *R bt* : 797 *r Bt* : 242 *r bt*
D/m für
9 : 3 : 3 : 1 = — 3,30 + 3,16 + 2,10 — 0,17
Crossoverwert = 46,6 ± 1,23 %.

Kreuzung Nr. 204; L. 2 aus Goldkönig × L. 6 aus Umbellatum. Koppelungsphase. 500 Samen gaben 426 Pflanzen.

Gefunden: 280 *R Bt* : 62 *R bt* : 50 *r Bt* : 34 *r bt*
D/m für
9 : 3 : 3 : 1 = + 3,97 — 3,71 — 2,22 + 1,48
Crossoverwert = 35,0 ± 2,99 %.

Kreuzung Nr. 231; L. 191 aus Witham Wonder × L. 422 aus Russische kleinsamige. Koppelungsphase. 500 Samen entwickelten 460 Pflanzen.

Gefunden: 291 *R Bt* : 54 *R bt* : 61 *r Bt* : 54 *r bt*
D/m für
9 : 3 : 3 : 1 = + 3,04 — 3,86 — 3,02 + 4,87
Crossoverwert = 29,8 ± 2,64 %.

Kreuzung Nr. 355; L. 232 aus de Winton × L. 451, *a, r, i, p, v, n, bt, o*. Koppelungsphase. 340 *N*-Pflanzen. % entwickelter Pflanzen = = 93,4.

Gefunden: 214 *R Bt* : 60 *R bt* : 36 *r Bt* : 30 *r bt*
D/m für
9 : 3 : 3 : 1 = + 1,69 — 1,60 — 2,15 + 2,58
Crossoverwert = 35,4 ± 3,37 %.

Kreuzung Nr. 433; L. 232 aus de Winton × L. 581, Stark panaschiert, *a, r, bt*. Koppelungsphase. 500 Samen haben nur 234 Pflanzen entwickelt. Semisterile Kreuzung.

Gefunden: 154 *R Bt* : 42 *R bt* : 21 *r Bt* : 17 *r bt*
D/m für
9 : 3 : 3 : 1 = + 2,95 — 0,31 — 3,84 + 0,64
Crossoverwert = 35,4 ± 4,05 %.

Kreuzung Nr. 473; L. 2 aus Goldkönig × L. 25, *A, ar, oh, R, Bt*. Koppelungsphase. 485 Samen haben nur 251 Pflanzen entwickelt. Semisterile Kreuzung.

Gefunden: 173 *R Bt* : 39 *R bt* : 28 *r Bt* : 11 *r bt*
D/m für
9 : 3 : 3 : 1 (korr.) = + 0,41 — 0,52 — 0,52 + 0,84
Crossoverwert = 42,3 ± 4,32 %.

Die beiden letztgenannten Kreuzungen zeigten keinen Zusammenhang zwischen Semisterilität und der Spaltung in *R* bzw. *Bt*.

Eine Zusammenfassung aller vorliegenden Ergebnisse von Spaltungen in den Genpaaren *A—R*, *A—Bt* und *R—Bt* ergibt, dass alle Zahlen für unabhängige Vererbung der Gene *A—R* und *A—Bt* sprechen.

Für *A—R* liegen Spaltungen mit etwa 28.000 Individuen vor, die keinerlei Koppelung anzeigen und für *A—Bt* gilt dasselbe, gestützt auf etwa 9.300 Individuen.

Anders liegen die Verhältnisse für das Genpaar *R—Bt*. Im Vorstehenden wurden alle mir bekannten Spaltungsverhältnisse für diese Gene zusammengestellt. Von diesen sind die von Kreuzungen, in denen gleichzeitig eine Spaltung in Kneifel- und Zuckererbsen (und z. T. auch in *N* : *n*) stattfindet, von geringerer Beweiskraft, da in diesen, wie früher erörtert worden ist, gewisse Klassifikationsschwierigkeiten in *Bt—bt* bestehen. Werden diese, RASMUSSONs beide Kreuzungen sowie meine Kreuzung Nr. 4 und 355, nicht berücksichtigt, so verbleiben insgesamt 14 Kreuzungen mit 5615 Individuen in der Koppelungsphase sowie 4 Kreuzungen mit 1243 Individuen in der Repulsionsphase. Für die 14 Kreuzungen ergibt sich:

Gefunden:	3452 *R Bt*	: 862 *R bt*	: 746 *r Bt*	: 555 *r bt*
D/m für 9 : 3 : 3 : 1 =	+ 7,90	— 6,53	— 10,49	+ 11,26

Crossoverwert = 35,4 ± 0,83 %. Für die 4 Kreuzungen in Repulsionsphase wurden erhalten 37,2 ± 1,58 %.

Dass wir es hier um eine vollkommen gesicherte Koppelung zwischen *R* und *Bt* mit einem Crossoverwert von etwa 36 % zu tun haben, steht ganz ausser Zweifel. Auch zeigen die Crossoverwerte eine gute Gruppierung um diesen Mittelwert. Meine zwei Kreuzungen mit Ausspaltung von Zuckererbsen zeigen auch dieselbe Koppelung an; die eine gibt ein Crossover von 35,4 %, die andere allerdings nur 46,6 %.

Mit Hinblick darauf, dass sowohl *A* und *R* wie *A* und *Bt* etwa 50 % Crossover zeigen, *R* und *Bt* dagegen einen vollkommen gesicherten Wert von etwa 36 %, erscheint es nicht zulässig auch das Gen *A* bzw. die Gruppe *A—Lf* (= *Xa*) an die Koppelungsgruppe *Pa—R—Tl—Bt* anzuhängen, wie dies v. ROSEN (1944, S. 311) getan hat. Es können nicht zwei Gene mit einem Crossover von 36 % in einem Chromosom liegen und überdies mit einem dritten im gleichen Chromosom gelegenen Gen je 50 % Crossover, d. h. unabhängige Vererbung aufweisen. Natürlich kann die Möglichkeit, dass es ein zweites *Bt*-Gen gibt nicht ausgeschlossen werden, aber die Annahme eines solchen erscheint erst berechtigt, wenn es durch Kreuzungen ohne Klassifikationsschwierigkeiten sicher nachgewiesen ist.

Die Aufteilung des Gens Cp in Cp_1 und Cp_2. — Gestützt auf die WELLENSIEKsche Koppelungsgruppe *Q*, *Cp*, *N* und *Bt*, deren Unhaltbar-

keit oben nachgewiesen worden ist, sowie mit Hinblick auf die von mir nachgewiesene Koppelungsgruppe *Gp—Cp—Fs—Ast* hat v. ROSEN (l. c.) das Gen *Cp* in zwei Gene Cp_1 und Cp_2 aufgeteilt. Auf Grund der oben vorgelegten Resultate erscheint diese Aufteilung unberechtigt und ist zu streichen.

Die grosse Koppelungsgruppe Nr. 1 von WINGE *D—P_2—O—I—Re—Fa—Z— — —Uni—M—Mp—F—B—St—P_1* (1936, Tab. 6). — Eine Kritik und Überprüfung der Unterlagen zu dieser grossen Gruppe ist hier umsomehr am Platz, als sie zwei der Gene enthält, die meiner vorstehend behandelten Koppelungsgruppe *N—Z—Fa—Td* angehören. Wie ersichtlich sind dies die Gene *Fa* und *Z*, für die früher auf Grund der bisher vorliegenden Ergebnisse ein Crossoverwert von etwa 18 % hat festgestellt werden können. WINGE scheint selbst nicht sicher zu sein, dass alle diese 14 Gene in einem Chromosom liegen, da er die Verbindung zwischen *Z* und *Uni* durch eine gestrichelte Linie angibt. Im Kommentar zu dieser Gruppe sagt er indessen, dass die Vereinigung dieser beiden Gruppen auf Grund der von ihm gefundenen Koppelung zwischen *Z* und *M* mit etwa 42 % Crossover erfolgt ist. Als Crossoverwert für die Koppelungsphase (1 Kreuzung) ergibt sich $43{,}7 \pm 2{,}94$ % und für die Repulsionsphase (2 Kreuzungen) $40{,}3 \pm 2{,}93$ %.

Es ist sehr bemerkenswert, dass WINGE die umfangreichen von H. und O. TEDIN (1928) über diese beiden Gene ausgeführten Untersuchungen hierbei nicht berücksichtigt. Diese erhielten folgende Spaltungen.

Gefunden: 362 *Z M* : 113 *Z m* : 135 *z M* : 49 *z m*
D/m für
9 : 3 : 3 : 1 = $-0{,}63$ $-1{,}08$ $+1{,}17$ $+1{,}27$
Crossoverwert = $48{,}0 \pm 2{,}85$ % (Koppelungsphase).

Gefunden: 402 *Z M* : 137 *Z m* : 129 *z M* : 55 *z m*
D/m für
9 : 3 : 3 : 1 = $-0{,}33$ $+0{,}35$ $-0{,}65$ $+1{,}27$
Crossoverwert = $46{,}8 \pm 2{,}69$ % (Koppelungsphase).

Gefunden: 5139 *Z M* : 1660 *Z m* : 1705 *z M* : 591 *z m*
D/m für
9 : 3 : 3 : 1 = $+0{,}49$ $-1{,}26$ $\pm 0{,}00$ $+1{,}00$
Crossoverwert = $51{,}0 \pm 0{,}78$ % (Repulsionsphase).

Schon diese Werte dürften genügen, um das Vorhandensein einer Koppelung zwischen *Z* und *M* in Frage zu stellen.

Selbst habe ich die Spaltung von *Z—M* in 9 Kreuzungen mit zusammen 4437 Individuen untersucht und in keiner auch nur eine Andeutung zu einer Koppelung gefunden. Die Crossoverwerte variierten von 46,0 bis 52,8 %. Insgesamt wurde erhalten:

Gefunden: 2522 *Z M* : 808 *Z m* : 864 *z M* : 243 *z m*
D/m für
9 : 3 : 3 : 1 = + 0,79 — 0,92 + 1,23 — 2,23
Crossoverwert = 48,3 ± 1,15 % (Repulsionsphase).

Aber es bedarf eigentlich gar nicht der Mitteilung dieser Zahlen, denn WINGE teilt in seiner eigenen Arbeit Spaltungen mit, die die Aufstellung seiner Koppelungsgruppe 1 nicht möglich erscheinen lassen. Er findet nämlich für die Genkombinationen *I—Z* 51,9 bzw. 48,4 %, für *I—M* 50,5 %, für *I—B* 49,6 %, für *B—M* 47,7 % und für *B—Z* 52,5 % Crossover. Es dürfte wohl ohne weiteres einzusehen sein, dass die vier Gene *I*, *Z*, *M* und *B*, die laut WINGEs Tab. 6 in etwa gleichen Abständen voneinander auf das Chromosom 1 verteilt sind, nicht in allen Kombinationen miteinander stets ca. 50 % Crossover geben können. Dies würde ja allen unseren Erfahrungen von Koppelungsstudien zuwiderlaufen und auch der MORGANschen Theorie über den Einfluss der linearen Anordnung der Gene in den Chromosomen auf das Crossover die experimentelle Grundlage entziehen.

Weitere Kommentare zu WINGEs Koppelungsgruppe 1 erscheinen damit wohl überflüssig. Sie zerfällt auf Grund der bisherigen experimentellen Daten in folgende drei Gruppen: *D—P_2—O—I—Re*, *Fa—Z* (zur Gruppe *N—Z—Fa—Td* gehörig) sowie *Uni—M—Mp—F—B—St—P_1*. Damit soll jedoch nicht gesagt sein, dass ich die Reihenfolge der Gene in der ersten und dritten Gruppe wie auch die Zugehörigkeit aller genannten Gene zu diesen Gruppen für bewiesen und richtig erachte. Die letztere soll zum Gegenstand einer nächsten Studie gemacht werden. Hier sei noch erwähnt, dass ich es auch noch als ganz unsicher betrachte, ob es zwei Gene für Purpurfärbung der Hülse, *Pur* (WINGEs P_1 und P_2) gibt.

G. v. ROSEN (1944, S. 310) hat die WINGEsche Koppelungsgruppe 1 übernommen und ihr noch WINGEs Gruppe 5, *S—Wb—K*, in nun geänderter Reihenfolge (*K—S—Wb*), angehängt. v. ROSEN begründet dies damit, dass in seinen »Artkreuzungen» zwischen *Pisum sativum* und *abyssinicum*, die für die Fertilität einen Gesamtmittelwert von 35,15 % gezeigt haben, eine lockere, ungefähr 40 % Crossover entsprechende Koppelung zwischen den Genpaaren *St—Wb*, *B—K*, *B—Wb*

und *F—Wb* gefunden worden ist, während die Genpaare *St—K* und *F—K* keine Koppelung gezeigt haben. Dass die Annahme einer Koppelung, wie die nun besprochene, mit Hinblick teils auf die Sterilität der Kreuzungen, teils auf die gefundenen Spaltungen nicht berechtigt erscheint, dürfte nicht besonders hervorgehoben zu werden brauchen. v. ROSEN deutet in seiner Tabelle 9 a allerdings an, dass die Beziehungen zwischen *K—S—Wb* sowie den drei von mir oben erwähnten Teilen der WINGEschen Koppelungsgruppe nicht sicher sind.

ZUSAMMENFASSUNG DER ERGEBNISSE.

1. Die Zähnung der Blättchen von *Pisum* und ihre Vererbung wurde studiert. Gezähnt dominiert über ungezähnt. Die Beurteilung der Zähnung erfolgte nach einer zehngradigen Skala, *td* und 1—9 (s. Fig. 1). *td*-Pflanzen haben nicht immer ganz ungezähnte Blättchen; nicht selten findet man den Zähnungsgrad 1, vereinzelt 2 oder 1—2 bzw. 1—3. Solche Pflanzen haben dann gewöhnlich auch zum Teil ganz ungezähnte Blättchen. Der Hauptstamm kann ungezähnte, die Verzweigungen Blättchen mit Zähnungsgrad 1—3 aufweisen. *Td*-Pflanzen haben gewöhnlich Zähnungsgrad 5 mit einer Variation bis zu 4 und 6, selten bis zu 3 und 7. Diese Verhältnisse müssen bei der genetischen Analyse berücksichtigt werden.

2. Auch Linien mit dem Zähnungsgrad 8—9 wurden beobachtet. Da sie unter *Td*-Pflanzen mit Zähnungsgrad 5 spontan ausgetreten sind, handelt es sich wahrscheinlich um eine Mutation, was stark dafür spricht, dass es von *Td* ein drittes Allel für die besonders starke und mehr sägeartige Zähnung 8—9 gibt.

3. In vier Kreuzungen wurde gesicherte Koppelung zwischen den Genen *N*, *Z*, *Fa* und *Td* festgestellt. Die mitgeteilten Spaltungsergebnisse berechtigen dazu, diese vier Gene hinsichtlich Ordnungsfolge und Abstände folgendermassen darzustellen: *N* — 15 % — *Z* — 18 % — *Fa* — — 28 % — — *Td*.

4. Die Beziehungen dieser Koppelungsgruppe zu anderen Genen bzw. Koppelungsgruppen wurde einer eingehenden und kritischen Überprüfung unterzogen, wobei sich folgendes ergeben hat.

5. Von den von WELLENSIEK (1925, 1928) und von WELLENSIEK und KEIJSER (1929) als miteinander gekoppelt angeführten Genen *Cp*, *Gp*, *Bt*, *N*, P_1, *V* und *Q* kann nur die zwischen *Gp* und *Cp* aufrecht erhalten werden. *N* gehört zur Gruppe *N—Z—Fa—Td*. Das Bestehen des Gens *Q*, das in dominanter Form Samenabort bedingen soll, wird

in Frage gestellt, da dies ebensogut durch partielle Sterilität im Zusammenhang mit strukturellen Unterschieden in den Chromosomen bedingt werden kann. Für die Übernahme der genannten WELLENSIEKschen Koppelungen oder eines Teils derselben in die Arbeiten von RASMUSSON (1927), WINGE (1936; Koppelungsgruppe 6) und v. ROSEN (1944; Chromosom III) gilt natürlich auch das eben Gesagte.

6. G. v. ROSENS (1944) kompilierte Koppelungsgruppe III besteht aus teils der von E. NILSSON (1939) vollkommen gesicherten Gruppe *Pa—R—Tl—Bt*, teils drei von WELLENSIEK übernommenen Genen (*Q*, *Cp* und *N*) sowie dem seit langem bekannten, gekoppelten Genpaar *A—Lf* (letzteres hier synonym mit *Xa* bezeichnet). Von v. ROSENS Gruppe III von 9 Genen kann nur einerseits E. NILSSONS Gruppe *Pa—R—Tl—Bt* und andererseits *A—Lf* aufrecht erhalten werden.

7. WINGES (1936) grosse Koppelungsgruppe 1 »*D—P*$_2$*—O—I—Re—Fa—Z—Uni—M—Mp—F—B—St—P*$_1$» soll laut WINGE selbst 4 auf dieses Chromosom ziemlich gleichmässig verteilte Gene (*I*, *Z*, *M*, *B*) enthalten, die in jeder Kombination etwa 50 % Crossover zeigen, d. h. unabhängig voneinander vererbt werden sollen! Die Unhaltbarkeit dieser Gruppe wird nachgewiesen. Sie zerfällt in drei Gruppen: *D—P*$_2$*—O—I—Re*, *Fa—Z* (zu *N—Z—Fa—Td* gehörig) sowie *Uni—M—Mp—F—B—St—P*$_1$. G. v. ROSEN (1944) hat WINGES Gruppe 1 übernommen und ihr noch die gut gesicherte Koppelungsgruppe *S—Wb—K* angehängt, was experimentell unbegründet erscheint. Die erste und letzte von WINGES Teilgruppen bedürfen hinsichtlich Genenfolge und teilweise auch Genzugehörigkeit einer experimentellen Überprüfung.

8. Da für die Aufteilung der Gene *Bt* in *Bta* und *Btb*, *Cp* in *Cp*$_1$ und *Cp*$_2$ sowie des Gens für Purpurfärbung der Hülse (*Pur*) in *P*$_1$ und *P*$_2$ noch keine sicheren experimentellen Grundlagen vorliegen, sollten diese einstweilen nicht mit Symbolen belegt worden sein; ihre weitere Verwendung soll unterbleiben.

SUMMARY.

(1) The inheritance of the dentation of the leaf-margins was studied. This dominant property was classified in accordance with a ten-graduated scale (*td* and 1—9; cf. Fig. 1). *td*-individuals are not always completely without dentation; they can also show dentation of the degree 1, rarely 1—2 or 1—3. This is especially the case on stem branches and on plants with low vitality. *Td*-plants have the degree of dentation 5 with a variation between 4 and 6, rarely with the degree

3 or 7. Knowledge of this phenomenon is of great importance for the genetical analysis. Pure lines with dentation 8—9 are also known. Since this extreme dentation has arisen by mutation, it is probable that a third allele to *Td* exists.

(2) In four crosses with altogether 2281 individuals linkage between the four genes *N*, *Z*, *Fa* and *Td* was ascertained. These genes show the following order and crossover values: *N* — 15 % — *Z* — 18 % — *Fa* — — 28 % — — *Td*.

(3) The relations between this linkage-group and other genes and linkage-groups were studied thoroughly with the following results. Of the linked genes *Cp*, *Gp*, *Bt*, *N*, P_1 and *Q* (linkage shown by WELLENSIEK, 1925, 1928, and by WELLENSIEK and KEIJSER, 1929), only *Cp—Gp* can be confirmed. *N* belongs to the linkage-group *N—Z—Fa—Td*. The existence of the gene *Q*, which as dominant should cause seed-abortion, seems to be doubtful, because structural differences in the chromosomes, now well known in *Pisum*, are also able to cause this phenomenon. The above-mentioned linked genes (WELLENSIEK) were also referred to in the papers of RASMUSSON (1927), WINGE (1936) and v. ROSEN (1944).

(4) The linkage group III with 9 genes, set up by v. ROSEN (1944), is made up of the established linkage-group *Pa—R—Tl—Bt* (E. NILSSON, 1939), the ancient linkage-group *A—Lf* and the genes *Cp*, *N* and *Q*, which are considered by WELLENSIEK to be linked.

(5) The large linkage-group *D—*P_2*—O—I—Re—Fa—Z—Uni—M—Mp—F—B—St—*P_1, established by WINGE (1936), contains four genes (*I*, *Z*, *M*, *B*), which should be distributed in the chromosome with very nearly the same interval between each other. According to WINGE they segregate in all combinations with about 50 % crossover! The instability of this linkage-group is demonstrated. It is to be divided into the following three groups: *D—*P_2*—O—I—Re*, *Fa—Z* (a part of *N—Z—Fa—Td*) and *Uni—M—Mp—F—B—St—*P_1. The large linkage-group of WINGE was included by v. ROSEN in his paper of 1944. To this group v. ROSEN, moreover, appends the well known linkage-group *S—Wb—K*, which is experimentally unfounded. The two linkage-groups *D—*P_2*—O—I—Re* and *Uni—M—Mp—F—B—St—*P_1 need a partial revision in respect of the order and inclusion of the genes.

(6) The division of the gene *Bt* into *Bta* and *Btb* (by RASMUSSON), of *Cp* into Cp_1 and Cp_2 (by v. ROSEN) and of the gene for the purple colouring of the pod (*Pur*) into P_1 and P_2 (by WINGE) is not yet

demonstrated experimentaliy, and on that account the use of this double symbols ought to be omitted for the present.

ZITIERTE LITERATUR.

1. HOSHINO, Y. 1915. On the inheritance of the flowering-time in peas and rice. — J. Coll. Agric. Tohoku Imp. Univ. Sapporo 6, 229—288.
2. IMMER, E. R. 1930. Formulae and tables for calculating linkage intensities. — Genetics 15, 81—98.
3. KAPPERT, H. 1925. Über die Zahl der unabhängigen Merkmalsgruppen bei der Erbse. — Z. f. ind. Abst. u. Ver. 36, 1—32.
4. LAMPRECHT, H. 1936. Genstudien an *Pisum sativum*. I. Über den Effekt der Genpaare *Con—con* und *S—s*. — Hereditas XXII, 336—360.
5. — 1937. Genstudien an *Pisum sativum*. II. Gestreifte Samenschale und ihre Vererbung. — Hereditas XXIII, 91—98.
6. — 1938. Über Hülseneigenschaften bei *Pisum*, ihre Vererbung und ihr züchterischer Wert. — Der Züchter 10, 150—157.
7. — 1941. Über Genlabilität bei *Pisum*. — Der Züchter 13, 97—105.
8. — 1942. Die Koppelungsgruppe *Gp—Cp—Fs—Ast* von *Pisum*. — Hereditas XXVIII, 143—156.
9. LAMPRECHT, H. und ÅKERBERG, E. 1939. Über neue Gene für die Ausbildung von Testafarbe bei *Pisum*. — Hereditas XXV, 323—348.
10. MENDEL, G. 1865. Versuche über Pflanzenhybriden. — Verhandl. d. naturforsch. Vereins in Brünn, IV, 3—47.
11. MEUNISSIER, A. 1918. Expériences génétiques faites à Verrières. — Extrait du Bull. Soc. nat. d'Acclimatation de France, 32 p.
12. NILSSON, E. 1929. Erblichkeitsversuche mit *Pisum*. I. Unterdrückung der Dominanz eines Faktors durch die Wirkung anderer genetischer Faktoren. — Hereditas XII, 17—32.
13. — 1939. Erblichkeitsversuche mit *Pisum*. X. Die Koppelungsgruppen *Pa—R—Tl—Btb* und *Wlo—P—Pl*. — Hereditas XXV, 48—64.
14. PELLEW, C. 1937. Linkage in structural hybrids in *Pisum sativum*. — Nature 139, 113.
15. RASMUSSON, J. 1927. Genetically changed linkage values in *Pisum*. — Hereditas X, 1—152.
16. — 1935. Studies on the inheritance of quantitative characters in *Pisum*. I. Preliminary note on the genetics of time of flowering. — Hereditas XX, 161—180.
17. ROSEN, G. v. 1944. Artkreuzungen in der Gattung *Pisum*, insbesondere zwischen *P. sativum* L. und *P. abyssinicum* BRAUN. — Hereditas XXX, 261—400.
18. STERN, C. 1933. Faktorenkoppelung und Faktorenaustausch. — Handbuch d. Vererbungswissenschaft. 19 (III).
19. SUTTON, A. W. 1913. Experiments in crossing a wild pea from Palestine with commercial peas. — 4th genet. conf. Paris, 1911. Bull. 3, 358—365.
20. SVERDRUP, A. 1927. Linkage and independent inheritance in *Pisum sativum*. — Journ. of Genetics 17, 221—251.

21. TEDIN, H. and O. 1928. Contributions to the genetics of *Pisum*. V. — Hereditas XI, 1—62; Plate I.
22. TSCHERMAK, E. v. 1919. Über Züchtung landwirtschaftlich und gärtnerisch wichtiger Hülsenfruchter. — Arb. d. D. Landw. Ges. für Österreich, Heft 4, 80—106.
23. WELLENSIEK, S. J. 1925. *Pisum*-crosses. I. — Genetica VII, 1—64.
24. — 1925 a. Genetic monograph on *Pisum*. — Bibliographia Genetica II, 343—476.
25. — 1928. *Pisum*-crosses. III. — Genetica XI, 225—256.
26. — 1929. The occurrence of more than 50 % crossing-over in *Pisum*. — Genetica XI, 509—518.
27. WELLENSIEK, S. J. and KEIJSER, J. S. 1929. *Pisum*-crosses. V. Inherited abortion and its linkage-relations. — Genetica XI, 329—334.
28. WINGE, Ö. 1936. Linkage in *Pisum*. — Comptes rendus d. trav. du Laboratoire Carlsberg. 21, 271—393.

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THE THEORY OF EVOLUTION FROM A BIOCHEMICAL POINT OF VIEW

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FROM a general biological point of view the term »evolution» might be interpreted as meaning the generation of forms of life which are better fitted than others to be the bearers of life. The theme has been treated as exhaustively as lies in the power of scientific research. But the great majority of thinkers have been captivated by the idea that just human beings are those forms of life which represent the highest refinement.

The possession of intense, psychic functions is regarded as something »higher», something better fitted for preserving the vital principle than the pure somatic functions. Thus, the whole anthropocentric attitude to the theory of evolution comes out in strong relief.

When I use the term »forms of life which are better fitted to be the bearers of life than others», this may need some further explanation. In a lecture (WIDMARK, 1932) I have discussed the possibility of life having arisen as a result of chance combinations of chemical processes. I came to the view that such constellations of reactions as can lead to the synthesis of living matter cannot be considered to occur or to have occurred. (By »living matter» I mean here the constellation of the chemical system of different substances which makes the whole capable of vital functions; cf. l. c., p. 4.)

If we set out from the conception that spontaneous generation is inconceivable from a chemical point of view, there only remain two explanations of the presence of life.

One is the existence of a creative power, a »*ratio*», which out of the manifoldness and variation-possibilities of syntheses selects and steers those which render possible the origin of living things. This line of thought leads to the religious acceptation of the existence of a creative power. Physical and chemical laws may certainly remain valid, but biogenetic chemistry is directed by an unknown laborant, whose existence we can only believe in without knowing anything about him.

The other explanation, which is perhaps as imbued with belief as the one just mentioned, is that the vital principle is a primary attribute that has not been created but has always existed. Living matter is a special form of existence of matter. It is »eternal», possibly transported from heavenly body to heavenly body, but constantly retains its »ratio» for the direction of chemical syntheses. This vital principle includes the conservation of itself as its greatest peculiarity. The perpetuity of living matter manifests itself in the most varying forms of existence. Change and transformation in different organisms are continually taking place, yet the »ratio» of the cells still goes on arranging for the continued existence of life, anxiously guarding against the risk of extinction. From a biological point of view living substance assumes varying shapes of existence. The most vital is the highest, without regard to the individual. Evolution, if such exists, has for its sole object the creation of the ideal vital substance.

The foundations of the evolutionary theory. — The history of the doctrine of evolution shows rather clearly that this theory has for the most part sprung from morphological observations. It has been framed by zoologists, botanists and geneticists.

The hypothetical chains of descent have been built up on comparative morphological observations of the different organisms and their organic systems. The comparisons have chiefly concerned the multicellular beings. Attempts have certainly been made to group the unicellular within certain regions of affinity, but here, too, observations of the morphological structure, the constitution of the organism, have been determinant. The morphologically more complicated forms of life have been generally denoted as the more developed, the higher. From the point of view of morphological and chemical differentiation I would here introduce the terms *monocytes* and *polycytes*. Certainly the term »monocytes» has previously been used as a designation for the freely existing cells in the blood tract of animals (white blood cells), but I consider it convenient and expedient to use this short term in this paper. Thus, by »monocytes» I mean unicellular organisms, by »polycytes» multicellular organisms for which previously we have employed such semi-general names as *Metazoa*.

Since, therefore, the theory of evolution is essentially based on morphology, it may be asked whether an evolution can be traced by a comparative study of the chemical system and chemical efficiency of the organisms.

An experiment of this kind might be carried out by disregarding

morphology and trying to arrange species on a chemical system. Should the chemical classification of the forms of life present a similar picture to that of the morphological, then the theory of evolution would be given a powerful support.

The perfect laboratory. — In an interesting article PIRIE (1938) has discussed the meaninglessness of the terms life and living. He advances the opinion that there is no sharp boundary between living and dead matter, just as little as between the colours yellow and green or between acid and alkaline reaction. The terms *life* and *living* »still have a very definite meaning when used by poets, knackers or soldiers, but little or none when used to describe the phenomena observed in tissue culture, virus research and kinetic studies on interrelated enzyme systems».

PIRIE considers it meaningless to speak of life and living because there is no definite dividing-line between living and dead matter. For my part, I have taken up an opposite standpoint (l. c., p. 4).

The frequently-advanced example of vira as something between living and dead matter would seem to be waning in effectiveness. Vira have no metabolism; they do not breath. They cannot be multiplied other than in living cells, and it is probable that it is just these which produce them. Chemically regarded, they are, at least in many cases, nucleoproteids. They can be crystallized, their »macromolecules» can be measured and photographed. It would seem meaningless to speak of life in this connection; rather the contrary.

I now come to the main point of my discourse. As soon as vital functions can be traced, chemical efficiency is complete and perfect. The chemical skill of the monocyte is as great as, often even greater than, that of the polycyte. Here, acquired chemical ability of an essential nature cannot be traced through the series of morphologically more or less complicated organisms. What we can observe is only specializations of certain reactions, which are already to be found in the lowest organisms.

Even the monocytes represent the perfect laboratory. They synthetize the specific protein of their species, and we cannot trace any systematic change to more and more complicated proteins either in the vegetable or animal world.

It might be questioned whether the formation of antibodies is not a recent acquisition, more especially in the Vertebrates. Our systematic knowledge in this field throughout the animal kingdom is as yet rather insignificant, however. Still, the fact that even the monocytes possess

the power of increasing their chemical defence against poisons is shown by, e. g., the newly acquired chemoresistance of the gonococci against sulphonamides. Here, perhaps, it is not a question of direct antibody formation in the ordinary sense. It is however presumable that synthetic ability to form toxins as well as antitoxins is already to be found fully developed in the monocytes.

So far as we know, all organisms can synthetize enzymes. The type and diversity of these enzymes vary with the needs of the organism. Thus, the ability to hydrolyze cellulose is principally found in certain bacterial strains. It is completely absent in the Vertebrates, is possibly present in some Invertebrates.

The utilization of glycides as a source of energy, the metabolism of the glycides in the organism in general, afford an instructive example of the conservatism of living matter. I would call to mind that the study of the utilization of monosaccharides, chiefly hexoses, by the yeast cells has given us the clearest explanation of how human muscle cells proceed in order to utilize such nutritive substances both aerobically and anaerobically. The processes are so alike in both yeast cell and man that we can speak, at most, of relatively insignificant variations, minor changes in an enzyme constellation. It is rather interesting to note that research in this field has been considerably facilitated by the very ready accessibility of monocytes, the yeast cells, as experimental material. It is simpler to perform experiments with a yeast culture as starting material than with mammalian muscle.

The occurrence of haemoglobin in the blood of the Vertebrates could possibly be interpreted as the result of an evolution to a higher mechanism of energy. The organic chemical complex, briefly described as an association of four pyrrol rings in a cycle, is however always traceable as a never absent constituent of living matter. This constellation of atoms seems to be primary. On account of the occurrence of these rings in almost all cells, from monocytes to polycytes, they are usually called cytochromes. Mostly the four pyrrol rings are found in combination with iron, in the form of blood pigments, but also with magnesium in chlorophyll and with copper in the respiratory proteins of certain Invertebrates.

It might be thought that higher psychical functions imply the presence of special substances that react in some special way at the exercise of these functions. No such substances have been observed. The chemical composition of the human brain is the same as that of any other brain. Difference in degree of psychical proficiency seems

rather to be bound up with morphological complexity of nerve tissue. The larger the latter is throughout the animal kingdom, the greater, on the whole, is the possibility of psychical performance.

The evolutionary theory considered from the standpoint that the biochemically most efficient organism is the most developed. — With this standpoint the matter has to be viewed against the background of the ability of the organisms to exist independently of accessory organisms.

Biochemically, we can in this respect seek out self-supporting organisms, such as have the most varied chemism, such as are not dependent on a more or less intimate symbiosis for the maintenance of their living substance.

If, following these lines, we make a search through the various phyla for the most perfect organisms, we must come to a stop at the chlorophyll-bearers, for only these are able to utilize the energy of light. Only these can convert matter into biogenic substance. Chlorophyll-bearing organisms are found already among monocytes (unicellular green algae, certain Flagellata). From a chemical point of view, accordingly, these are more independent, more perfect, than the more complex animal polycytes, morphologically the higher standing. From this point of view man is an incompletely developed organism. He is a parasite on cryptogams and phanerogams, just as the toothwort is on the roots of the hazel. He has no independence: is without the ability to collect cosmic energy in his vital laboratory, to synthetize manifold essential biogenic substances. He is doomed to be a parasite on the chlorophyll-bearers, to be an organism devoid of the most important chemical property of living matter. It is difficult to see any evolution in this from a biochemical point of view.

This lack of synthetic capability comes to the fore in the form of vitamin needs. Many monocytes can create, for instance, vitamins A, B and C, but we human beings cannot do so.

Phylogeny and ontogeny. — If biochemical observations are to give any support for the evolutionary theory, we must find some harmony between the generation of species and of individuals from a chemical point of view.

What has been set forth above will show that I cannot find any biochemical evidence in favour of the concept »evolution» in a phylogenetic respect.

This concept is rather perplexing in the light of the fact that a necessary condition for the development of animal life is the pre-

existence of organisms which in a chemical respect are the highest developed, the chlorophyll-bearers.

To ensure a logically safe foundation for the doctrine of evolution it would seem justifiable to demand that from the point of view of morphological research ontogeny and phylogeny should possess some affinity with biochemical observation. Would a biochemist, without taking morphology into account and solely for chemical reasons, have need of making use of the terms ontogeny and phylogeny?

What is now wanted is to try to find some chemical proofs that phylogenetical evolution has something in common with changes in the chemism of the growing individual.

Certain observations may be set forth. The power of regeneration diminishes during embryonic development, just as it seems to diminish in the phylogenetic series. The possibility of transplants from individual to individual diminishes from the embryo to the adult stage, just as is the case in the morphologico-evolutionary series. It is questionable whether this isolation is something tending to a higher stage. Rather would it seem to be a limitation in chemical efficiency.

Evolution and evolutionary mechanism. — The monocytes — provided there does not exist any form of symbiosis with or parasitism on other organisms — live their independent lives. They possess no activity of their own by which they can influence neighbouring cells in respect of function and organization. Quite different is the case with the polycytes. Here, according to reliable experimental experience (for the literature, see NEEDHAM, 1942), there exists an intimate chemical co-operation between the cells. The »formative» functions enter into activity. It is however of importance to recall that we can scarcely trace, phylogenetically or ontogenetically, any essential new formation in a chemical respect from the primitive cell-states to the more complex. Formative stimuli are already found in pronounced form among hydrozoa. The co-operation between the cells is so organized that there is reason to postulate that every somatic cell of these organisms contains within it those developmental resources which can form the special cell formations for the organism.

Formative stimuli appear to be already complete in the egg of the echinoderm, a fact shown by exhaustive tests. Ontogenetically, it is interesting to note that already after the first cleavage the ovum of the frog distributes the evocators between the two new cells, that each of them has the power of growing into a new, complete individual. Not until after gastrulation does there appear a more differentiated growth

mechanism, so that different cells can no longer grow into entirely new individuals, but only into definite organic parts.

According to NEEDHAM's terminology (l. c., p. 686), an evocator is a chemical substance emitted by the organisator, which is a living part of the as yet incomplete individual (»a living part of an embryo, which exerts a morphogenetic stimulus upon another part or parts, bringing about their determination and the following histological and morphological differentiation»).

Respecting the chemical constitution and biological originality of these substances we still know nothing. There are no facts to indicate that the polycyte presents anything new in a chemical respect by the origin of evocators with chemical properties foreign to the monocytes.

To some extent the same reasoning must be applied when considering hormones and vitamins. The presence of hormones is a chemically necessary condition for the internal organization of the polycyte commonwealth, just as are the hypothetical evocators for its growth. Hence the question can be viewed from the standpoint that it is the morphological complexity which first creates the need of these intermediary chemical substances. It is the individual cells which are the synthetic laboratories. The production of hormone is secondary to morphological conformation. Of course, it may be reasoned that just this specialization of the cells for hormone production is an evolution. Through evolution the cells in LANGENHAN's islands have acquired a new faculty, the art of producing insulin, and the cells in the adrenal medulla the art of elaborating adrenalin, by which the carbon hydrate metabolism of the polycyte is regulated. Even the specific bacterium that brings about the characteristic fermentation of cabbage into sauerkraut produces, however, acetylcholine, that tissue hormone so important for nervous activity.

In this connection it is of some importance to note that the root tubercles of podded plants, which are of such fundamental importance for the world's nitrogen conversion, can, according to THIMANN (1936), only function because the symbiotic bacteria produce auxins for their host.

Different organisms vary so immensely in their dependence on vitamins and their ability to synthetize these substances that it is difficult to reconcile their chemical properties with evolution.

The evolution of atoms. — The assumption has been advanced that hydrogen is the primary element of matter, from which heavier and heavier atoms have been formed. Astronomers, for instance, would

seem to have shown that the chemical elements undergo perpetual »development» towards higher and higher atomic weights. On our earth we have not arrived further than to the weight 238, uranium, but on other masses of matter in the universe the occurrence has been established of atoms having a weight of 30,000 or possibly double this. It is possible that in the interior of stars atoms of extremely high atomic weights are created — provided we can speak of atoms under such special conditions.

We may wonder whether this tendency to higher atomic weights can in any way harmonize with the theory of the evolution of living matter. It is of the greatest importance here to be able to record the fact that living matter in all its varying forms only works with comparatively low atomic weights. The chemism of practically all life and the composition of living matter are restricted to atomic weights under 60 (hydrogen 1, carbon 12, nitrogen 14, oxigen 16, sodium 23, magnesium 24, phosphorus 31, sulphur 32, potassium 39, calcium 40, iron 56). Only in exceptional cases and in extremely small quantities do such heavy atoms occur as copper (63), bromine (80), iodine (126). The heavy atoms are incompatible with vital processes, poisons: antimony 122, barium 137, platinum 195, gold 197, mercury 201, lead 207, bismuth 209, radium 226 and uranium 238. We can therefore straightaway dismiss the idea that a higher atomic weight should be of higher value than a lower. On the contrary, as a rule atomic weights above 60 are hostile to life.

Nor has it been possible to discern any signs that living matter in any way prefers heavy isotopes to others (deuterium 2 as against hydrogen 1, heavy nitrogen 15 as against nitrogen 14).

Accordingly, from an evolutionary point of view, there is nothing to glean from the standpoint: »high and low atomic weight».

If, therefore, there is an »evolution of atoms», it must glide hastily past the conditions necessary for the existence of life.

LITERATURE CITED.

1. NEEDHAM, J. 1942. Biochemistry and morphogenesis. — University Press, Cambridge.
2. PIRIE, N. W. 1938. The meaninglessness of the terms life and living. — Perspectives in Biochemistry, Cambridge.
3. THIMANN, K. V. 1936. On the physiology of the formation of nodules on legume roots. — Proc. Nat. Acad. Sci. (Washington), 22, 511.
4. WIDMARK, E. M. P. 1932. Livets uppkomst genom uralstring. — Kungl. Fysiograf. Sällsk. i Lund Förh., Bd. 2, Nr. 11.

HYBRID VIGOUR IN CROSSES BETWEEN PURE LINES OF GALEOPSIS TETRAHIT

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SINCE 1924 numerous crosses between pure lines of the self-fertilizing species *Galeopsis Tetrahit* have been undertaken. These crosses were made in order to study the genetics of flower colour differences and the degree and inheritance of the partial sterility frequently met with in the F_1 hybrids (cf. MÜNTZING, 1929, 1930, 1932, 1937, 1938). During this work it was often observed that the F_1 hybrids had quite a good vigour, and sometimes they seemed to surpass both parent lines in this respect. Therefore plant height in hybrids and parent lines was measured, this work being undertaken at the end of the summer when the plants had reached their maximal size. Plant weight would have been a better index of vigour, but as the plants had to be preserved for other purposes, only the height values could be determined. However, there is certainly a rather strong positive correlation between weight and height and, thus, the latter character is a rather reliable measure of plant vigour.

In many cases the number of F_1 plants was too low to be of any use for the present problem. However, looking through the height data from the years 1928—1942 a total of 24 different cross combinations were found to be available, in which the number of F_1 individuals of each combination amounted to at least 10 plants. The number of individuals in the parent lines grown at the same time was also at least 10 and generally higher. More accurately, the number of F_1 plants in the 24 crosses ranged from 10 to 39 with an average number of 16,8. In the parent lines the corresponding number ranged from 10 to 93, the average value being 20,5.

The F_1 and parental seeds were germinated at the same time, the seedlings placed in boxes and later on transplanted to the field. In the field-plots the plants were grown in rows of four, the distance between plants as well as rows being 30 cm. The F_1 plants were always planted close to the female parent line. The male parent line was grown in the same small field at a variable distance from the F_1 plots.

If F_1 plants of both reciprocal combinations were available they were grown together close to one of the parents.

The following 14 pure lines are involved in the crosses: A, B, C, D, E, F, G, I, J, L, M, S, »yellow *Tetrahit*» and »Cheviot». The last-mentioned line was collected in 1939 in the Cheviot Hills, North England. Most of the other lines have been in culture since 1925. Their appearance and origin have been described in previous publications [1] (MÜNTZING, 1930, 1932, 1937, 1938).

TABLE 1. *Plant height in F_1 and parent lines.*

Average plant height in cm.			F_1 height (relative values)	Pollen percentage in F_1
Cross combinations	Parent lines			
A × X: 71,2	A: 54,5	X: 49,7	137	71
A × J: 86,7	A: 63,1	J: 84,0	118	79
A × D: 82,2	A: 61,8	D: 81,1	115	94
B × G: (1935) 95,8	B: 76,3	G: 100,7	108	87
» (1937) 125,7	B: 84,0	G: 117,4	125	87
B × C: (1936) 111,9	B: 112,7	C: 90,0	110	57
» (1937) 94,4	B: 84,0	C: 66,1	126	57
B × X: 92,9	B: 84,0	X: 49,7	139	54
C × Cheviot: 107,5	C: 106,7	Chev.: 66,1	124	50
C × J: 65,0	C: 66,1	J: 56,9	106	94
C × M: 94,2	C: 66,1	M: 85,0	125	54
C × E: 69,3	C: 61,7	E: 69,2	106	94
C × X: 64,0	C: 61,7	X: 54,5	110	94
C × D: 85,0	C: 64,2	D: 81,0	117	79
C × I: 73,4	C: 61,7	I: 85,5	100	67
D × I: 126,5	D: 111,0	I: 100,5	120	73
D × M: 112,1	D: 111,0	M: 85,0	114	94
D × Cheviot: 108,3	D: 92,6	Chev.: 106,7	109	90
F × L: 85,7	F: 68,0	L: 88,5	110	64
F × X: 68,0	F: 71,1	X: 54,5	108	98
G × Cheviot: 113,1	G: 106,6	Chev.: 106,7	106	90
G × X: 74,1	G: 83,8	X: 54,5	107	95
I × J: 70,0	I: 85,5	J: 45,4	107	56
J × M: 66,4	J: 46,3	M: 77,7	107	75
L × Cheviot: 108,1	L: 99,5	Chev.: 106,7	105	90
X × Cheviot: 88,1	X: 37,7	Chev.: 106,7	122	50

The height measurements of the 14 F_1 combinations and their parents are given in Table 1. For each F_1 combination the data of

[1] In these papers the denominations T—A, T—B, T—C, etc. correspond to A, B, C, etc. in the present paper.

both reciprocal cross combinations are combined if the cross has been made in both directions. In the cross combinations B × G and B × C data are available from two different years. The relative values (in the second column from the right) give the F_1 heights in per cent of the parental average values. Thus, for example, the F_1 combination A × X had the average height 71,2 cm., the corresponding values of the parent lines being 54,5 and 49,7 cm. respectively. The mean value of the parents will be 52,1, and if this value is considered to be 100 the F_1 height will be 137.

According to the table not a single F_1 value is below the average of the parents, the relative values ranging from 100 to 139. In one combination, C × I, the F_1 plants show the same height as the average of the parents, in all the other cases they surpass this average. In 16 cases of 26 the F_1 plants were taller than both parents, in nine cases they were taller than the average of the parents but did not reach the value of the taller parent. The latter category is chiefly represented by cases involving a strong difference in height between the two parent lines.

Though the complication of line differences in earliness has not been taken into consideration, the data presented undoubtedly demonstrate that the F_1 plants show a certain degree of hybrid vigour. On an average the F_1 combinations measured were 14,6 per cent taller than the mean value of the parents.

As the number of F_1 plants in the majority of cases was somewhat lower than the number of plants in the plots of the parent lines, there may be a suspicion that the F_1 plants had been slightly favoured owing to less crowding at the time of germination and in the seedling boxes. However, in 7 cases of the 26 the number of F_1 plants was higher than that in both parent lines. In these cases the relative height values of the F_1 plants ranged from 105 to 139 with an average of 114,7. Thus, these seven cases showed just the same degree of hybrid vigour as the other cross combinations. This demonstrates that the lower average number of the F_1 plants has not influenced their average height. The rather good reliability of the average values obtained is further indicated by four cross combinations, in which a rather high number (more than 10) of maternal plants were obtained together with the F_1 plants. These maternal plants, which appeared in consequence of a partially unsuccessful crossing technique, had evidently been treated in exactly the same way as the F_1 plants derived from the same seed sample. If the hybrid vigour of the F_1 plants were only apparent and

due to some kind of favouring of the F_1 plants unshared by the line plants, the maternal plants occurring among the F_1 individuals should also be more vigorous than the plants of the female parent line. This was not so, however, the maternal plants in three cases of the four having slightly lower values than the female parent line. The four pairs of values (maternal plants and female parent respectively) were the following: 109,0—106,7; 105,9—106,6; 88,5—99,5; 100,0—106,7. Only in the first pair is the average of the maternal plants slightly but insignificantly higher than that of the female parent line.

The material is too small to decide whether the different lines are equivalent as inducers of hybrid vigour. It seems most plausible, however, that the causes of this vigour are not concentrated to special lines but are rather evenly distributed.

TABLE 2. *Correlation between degree of sterility and degree of hybrid vigour in* F_1.

Percentage of good pollen	Degree of hybrid vigour (F_1 height, relative values) 100	105	110	115	120	125	130	135	140	n	M
50		1	—	1	2	1	—	1		6	122,5
60	1	—	1							2	—
70		1	—	2	1	—	—	1		5	120,5
80				1						1	—
90		7	2	1						10	109,5
100											

As shown in detail previously the pure lines of *Galeopsis Tetrahit* are perfectly fertile, but the F_1 hybrids are frequently partially sterile. Of 66 combinations tested (MÜNTZING, 1938), 30 had 90—100 per cent good pollen, like the parent lines, but the other 36 combinations were partially sterile with a pollen percentage ranging from 50 to 89. Of the 24 cross combinations now examined, 14 are partially sterile and 10 fertile with 90—100 per cent good pollen. The percentage of good pollen for each combination is given in Table 1 (to the right).

Interesting enough, a correlation was found between hybrid vigour and pollen fertility, the partially sterile F_1 combinations on an average showing a higher degree of hybrid vigour than the fertile F_1 combinations. This is evident from Table 2 and the calculations based on these data. In the 10 fertile F_1 combinations the average vigour value

(F_1 height in per cent of the average height of the parents) is 109,50, the corresponding value of the 14 partially sterile F_1 combinations being 119,29. Testing the distributions with an analysis of variance and a working mean of 112,50, the following values were obtained:

	Degrees of freedom	Sums of square	Mean square
Between series	4	36,3583	9,0896
Within »	19	45,6000	2,4000
Total	23	81,9583	—

The v^2 value (9,0896 : 2,4000) will be 3,79, which corresponds to a P intermediate between 0,05 and 0,01.

The coefficient of correlation was also calculated for the same data and found to be — 0,50. The significance of this coefficient was tested by using the formula $t^2 = \frac{(n-2)r^2}{1-r^2}$. This gave a t of 2,74 and a P intermediate between 0,01 and 0,02. Thus, the significance of the observed correlation is rather good, and hence it is highly probable that the partially sterile F_1 combinations really show a higher degree of hybrid vigour than the fertile F_1 combinations. Moreover, this correlation is probably gradual, the most sterile class of F_1 combinations also showing the highest degree of hybrid vigour (122,5 per cent).

The reason of this rather interesting correlation is not clear. The most plausible explanation seems to be that there is a positive correlation between the degree of genetic diversity and the degree of structural chromosome differentiation. Evidence has been obtained that the latter phenomenon is responsible for the partial sterility observed (cf. MÜNTZING, 1938).

It might be suggested that the greater vigour of the partially pollen sterile F_1 generations is due to a physiological correlation between vigour and sterility. In the partially sterile F_1 generations seed setting is also slightly reduced and therefore more energy might be left for vegetative growth. This is rather improbable, because plant growth seems to be finished with the development of the terminal inflorescense already before the seeds are ripe. It would be desirable, though, to test this point accurately by making repeated measurements during the whole period of development.

It is of interest to compare the hybrid vigour of the intraspecific *Tetrahit* hybrids with the increased vigour characteristic of the hybrids

between *Galeopsis Tetrahit* and *bifida*. According to data previously published (MÜNTZING, 1930, pp. 258—263), four different cross combinations between *Tetrahit* and *bifida* showed quite a pronounced hybrid vigour in F_1. In all of these four combinations plant height in F_1 surpassed the parental average by about 20 per cent. This F_1 vigour is quite obvious even at an early stage and is probably not increased by the partial sterility of the mature hybrids.

The hybrid vigour in the line crosses in *G. Tetrahit* is probably of essentially the same kind as in the *Tetrahit* × *bifida* crosses though, on an average, less pronounced. In this connection it should be remembered that *G. Tetrahit* and *bifida* sometimes intercross in nature and, thus, all *Tetrahit*- and *bifida*-lines may ultimately be derived from such spontaneous hybrids. However, all pure lines of *G. Tetrahit* used for the crosses were morphologically quite typical and sharply separated from the *bifida* lines.

In spite of the very extensive literature on inbreeding and hybrid vigour relatively little has been published about vigour in F_1 hybrids between pure lines of strictly self-fertilizing plant species. However, some data of this kind have been gathered in tomatoes, beans, peas, and self-fertilizing cereals. Hybrid vigour in *Solanum lycopersicum* has been observed by several workers. A review of these cases is given by DASKALOFF (1938) together with an account of his own rather extensive experiments. Hybrid vigour occurred in all cross combinations produced, the increases in yield ranging from 5 to 37 per cent with an average of about 20 per cent. Reciprocal crosses gave the same result. In three crosses DASKALOFF also found the yield to be slightly but significantly lower in F_2 than in F_1.

In *Phaseolus vulgaris* WINGARD (1927) studied 5 cross combinations between different varieties. The F_1 plants of all the crosses were more vigorous and produced more pods than either parent. MALINOWSKI (1928, 1935) also made several crosses within the same species. Only two of these crosses are said to give luxuriating F_1 hybrids. One of these combinations was studied in detail, and in this case the F_1 heterosis was very strong. The F_1 plants were found to react very much to a change in day-length. Hence the author concludes that we have to deal here with a change of the degree of photoperiodic response caused by special factors separated in the parental forms and united in F_1.

Some evidence of hybrid vigour has also been obtained in *Pisum sativum*. RASMUSSON (1927) observed that certain heterozygotes gave

seeds with a higher percentage of germination than the corresponding homozygotes, and NICOLAISEN (1932) found that seeds produced by crosses between pure lines were considerably larger than the average of the parent varieties.

As demonstrated by H. and O. TEDIN (1926) the difference in type of spike between 6-rowed and 2-rowed barley is in the main caused by a single pair of factors, *Z—z*. Disregarding the complicating effect of a special height factor *H*, linked with *Z*, the heterozygous *Zz* plants were found to be more vigorous than both homozygotes, *ZZ* and *zz*. According to the authors, this may be either a specific effect of the heterozygosity or due to linkage between *Z* and *z* with minor factors for plant height.

Hybrid vigour in oats has been reported by COFFMAN and WIEBE (1930), studying 21 crosses between standard varieties, and by COFFMAN and DAVIS (1934)[1]. A thorough investigation of hybrid vigour in *Triticum vulgare* was made by ENGLEDOW and PAL (1934). The cross Little Joss × Thule was studied in detail and 56 other *vulgare* crosses on a smaller scale. In most of the crosses the F_1 had a greater tillering capacity and yield than the average of the parents. In the cross Little Joss × Thule the increased vigour in F_1 was observed to be followed by a decrease in F_2 and a further decrease in F_3. In the same cross the two reciprocal F_1 combinations were significantly different in vigour. In F_2 there were slight indications of difference, whereas the F_3's were identical in vigour. On account of this reciprocal difference the authors find it difficult to accept a factorial explanation of hybrid vigour. However, the cause of this unexpected difference may be a maternal effect of the Thule parent, which according to the tables is much inferior to Joss in tillering capacity as well as yield of grain.

From an examination of all the published cases of hybrid vigour ENGLEDOW and PAL (l.c.) conclude that the phenomenon occurs preponderantly in crosses among parents which are (a) diploid, (b) naturally out-pollinating and (c) endospermic in seed. The present case in *Galeopsis Tetrahit* is an exception to this rule in all three respects, the species being tetraploid, strictly self-fertilizing, and the seeds non-endospermic. As far as I know, it is also the first wild species in which hybrid vigour has been observed to occur in crosses between pure lines.

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[1] These papers are only known to me from the Resumptio Genetica. Under the present circumstances it has not been possible to procure the original papers.

LITERATURE CITED.

1. COFFMAN, F. A. and DAVIS, L. L. 1934. Heterosis or hybrid vigour in oats. — Journ. Amer. Soc. Agron., Vol. 26, pp. 318—327.
2. COFFMAN, F. A. and WIEBE, G. A. 1930. Hybrid vigour in oats. — Journ. Amer. Soc. Agron., Vol. 22, pp. 848—860.
3. DASKALOFF, CHR. 1938. Beitrag zum Studium der Heterosis bei den Tomaten in bezug auf die Herstellung von Heterosis-Sorten für die Praxis. — Die Gartenbauwissenschaft, Bd. 11, pp. 129—143.
4. ENGLEDOW, F. L. and PAL, B. P. 1934. Investigations on yield in cereals. VIII. Hybrid vigour in wheat. — Journ. Agricult. Sci., Vol. 24, pp. 390—409.
5. MALINOWSKI, E. 1928. A peculiar case of heterosis in *Phaseolus vulgaris*. — Zschr. f. ind. Abst.- u. Vererb.-lehre, Suppl.-Band 2, pp. 1090—1093.
6. — 1935. Studies on hybrid vigour in *Phaseolus vulgaris* (L.) SAVI. Part I. Vigorous sizes and photoperiodic response of the F_1 plants. — Zschr. f. ind. Abst.- u. Vererb.-lehre, Bd. 70, pp. 96—124.
7. MÜNTZING, A. 1929. Cases of partial sterility in crosses within a Linnean species. — Hereditas XII, pp. 297—319.
8. — 1930. Outlines to a genetic monograph of the genus *Galeopsis*. — Hereditas XIII, pp. 185—341.
9. — 1932. Disturbed segregation ratios in *Galeopsis* caused by intraspecific sterility. — Hereditas XVI, pp. 73—104.
10. — 1937. Multiple allels and polymeric factors in *Galeopsis*. — Hereditas XXIII, pp. 371—400.
11. — 1938. Sterility and chromosome pairing in intraspecific *Galeopsis* hybrids. — Hereditas XXIV, pp. 117—188.
12. NICOLAISEN, W. 1932. Über quantitative Xenien bei Roggen und Erbsen. — Zschr. f. Züchtung, Reihe A., Pflanzenzüchtung, Bd. 17, pp. 265—276.
13. RASMUSSON, J. 1927. Genetically changed linkage values in *Pisum*. — Hereditas X, pp. 1—152.
14. TEDIN, H. and O. 1926. Contributions to the genetics of barley. I. Type of spike, nakedness and height of plant. — Hereditas VII, pp. 151—160.
15. WINGARD, S. A. 1927. The immediate effect of crosspollination on the size and shape of bean seed. — Genetics, Vol. 12, pp. 115—124.

A HAPLOID SUGAR BEET AFTER COLCHICINE TREATMENT

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IN the summer of 1942 numerous colchicine treatments of shooting sugar beets were performed in the greenhouses of the Hilleshög Beet Breeding Station of the Swedish Sugar Co. The c_1 seeds, resulting from these treatments, were put for germination in small fixing pots during the following winter. The chromosome numbers of the plants were determined at the Cyto-genetic Laboratory of the Swedish Seed Association, Svalöf. As is usual after such treatments, diploid, triploid as well as tetraploid c_1 plants were obtained. In one number studied, C 3841, besides these chromosome numbers, there also occurred one haploid plant with somatically 9 chromosomes (C 3841—41). The colchicine treatment had probably damaged one of the gametes involved. One pollen grain, for instance, may have been able to stimulate embryo development although incapable of fertilization. Although the chromosomes of several thousand c_1 seedlings of sugar beets and other plants have been studied at this laboratory during the last few years, the present instance is the first case of haploidy found after colchicine treatment. Since it seems to be the first case on record of haploidy in sugar beets, special attention has been directed to the morphology and cytology of this haploid sugar beet plant.

I am very much indebted to Miss MAGNA PALM, Svalöf, for making the slides for this investigation.

I. MORPHOLOGY.

The number C 3841 was deposited for germination in February, 1943, and the haploid was identified in April. During summer and autumn it was grown together with $2x$, $3x$ and $4x$ sister plants in pots in the greenhouse. Its general appearance was studied continually and compared with the sister plants. It differed from them in being markedly tender. It had more numerous leaves than the sister plants. On some occasions the numbers of leaves were counted ($2x$, $3x$ and $4x$ are means of two plants of each class):

Date:	21/4	5/5	24/5	16/6	14/7	12/8
x:	13	14	19	29	43	56
$2x$:	10	14	16	25	35	38
$3x$:	10	15	19	22	29	39
$4x$:	7	11	13	18	22	31

Thus, the haploid had, especially towards the end of the season, decidedly more leaves than average diploids. The tetraploids, on the other hand, had a lower number of leaves than the diploids throughout the vegetative period. As already earlier demonstrated on a more representative material (LEVAN, 1942 b), the leaf number of first year sugar beets follows the general viability curve: $2x$, $3x$ and $4x$ plants have more leaves than the aneuploids. Among the euploids the diploids have the most leaves, while triploids and tetraploids have fewer. On that occasion (l. c., Table 2, p. 352) the three euploid numbers had on an average 22,3, 18,8 and 15,0 leaves per plant respectively.

The shape of the leaf lamina in the haploid was long and narrow. The leaf index (length : breadth) of the material was as follows:

Date:	21/1	5/5	24/5	16/6	14/7	12/8	Mean
x:	2,5	2,0	1,5	1,4	1,4	1,5	1,7
$2x$:	1,4	1,4	1,3	1,4	1,4	1,7	1,4
$3x$:	1,2	1,3	1,3	1,2	1,2	1,3	1,3
$4x$:	1,4	1,1	1,4	1,3	1,3	1,3	1,3

The index of the haploid was almost all the time somewhat higher than in the comparative material. The haploid belonged to the type of sugar beet plants to which I directed attention in my above-mentioned paper (l. c., p. 357) and which is characterized by many small leaves, narrow and long in shape. They were especially characteristic of the chromosome numbers between $2x$ and $3x$, which were of a low viability, and it was concluded that this beet phenotype may be taken as an indication of lacking vitality.

Although the haploid was decidedly weaker than the control plants, it formed a beet root of considerable size (about 2 cm in diameter) during the summer. Later on in the autumn, when the plants began to show signs of maturity, they were no longer watered, and in November they were placed in a greenhouse under a constant temperature of + 2 centigrades. They were left here until the 20th of January, 1944, when they were moved into a heated greenhouse. Already about the 1st of February shooting commenced in the haploid, rapidly followed

by shooting also in the controls. Thus, shooting was somewhat earlier in the haploid, and this advance of a couple of days shown by the haploid was maintained during all the flowering period. The first flower opened on the following dates (order x, $2x$, $3x$, $4x$): $^{25}/_{2}$, $^{26}/_{2}$, $^{1}/_{3}$, $^{2}/_{3}$. General flowering was noted on the following dates: $^{1}/_{3}$, $^{28}/_{2}$, $^{4}/_{3}$, $^{1}/_{3}$. In the flowering stage also the haploid was more slender than the other plants (Fig. 1). It was also lower in height. The plant height was measured on the following two occasions:

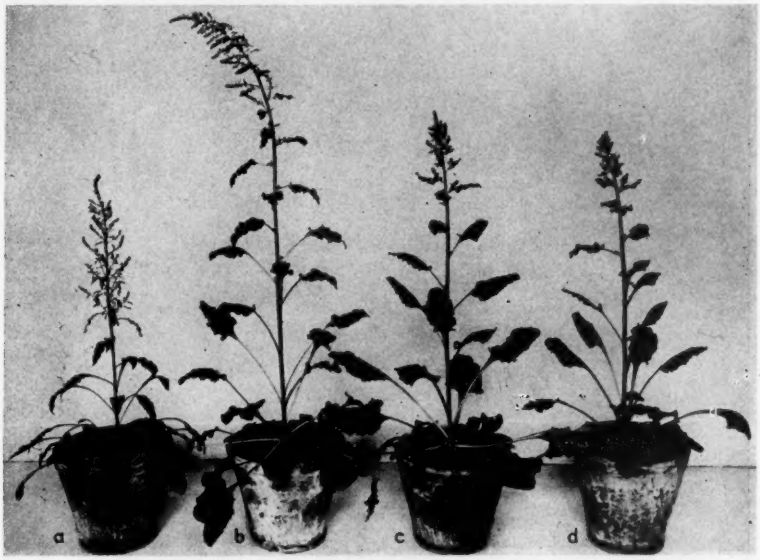

Fig. 1. General appearance of the sugar beet haploid, *a*, as compared with diploid, *b*, triploid, *c*, and tetraploid, *d*, sister plants.

Date:	$^{25}/_{1}$	$^{16}/_{8}$
x:	53	69
$2x$:	91	112
$3x$:	69	100
$4x$:	63	90

The haploid was the lowest and the diploids the highest. The haploid formed, however, a normally developed inflorescence that was quite as rich as the others. The general type of the haploid in the flowering stage was normal and well within the variation limit of ordinary diploid

sugar beets. If it had grown in a seed field, it would hardly have been detected. As the flowering proceeded, the branches of the haploid kept

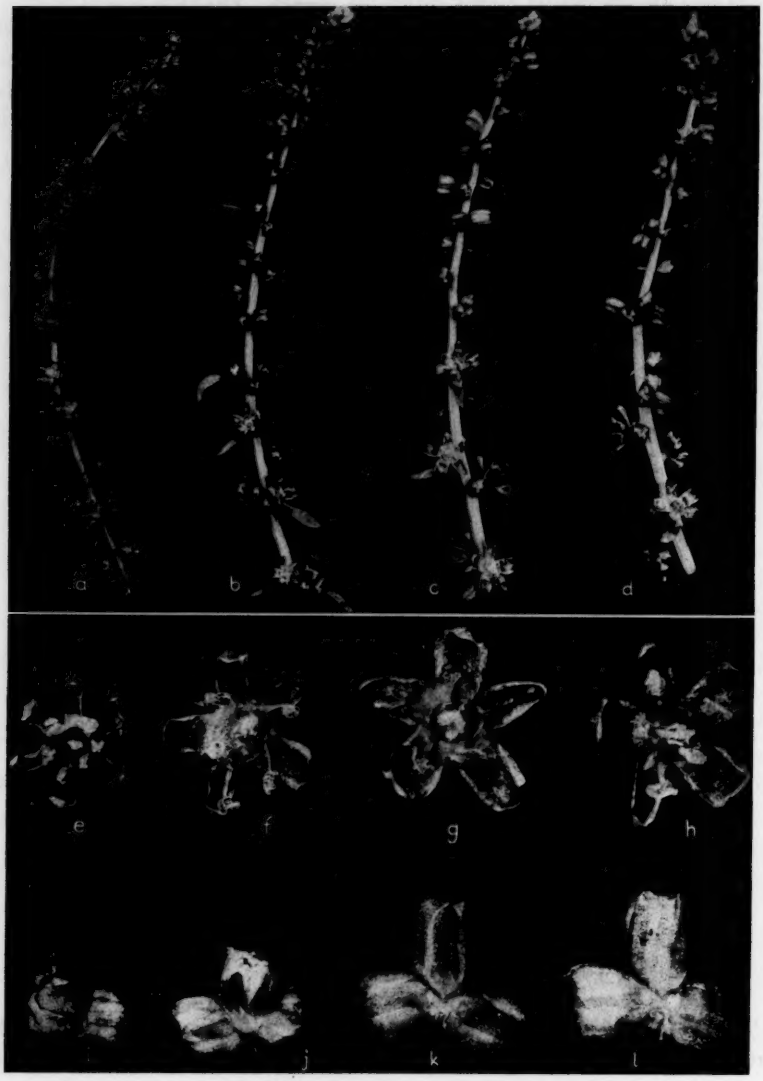

Fig. 2. Flowering branches (*a—d*), flowers (*e—h*) and buds (*i—l*) of haploid, diploid, triploid and tetraploid (in order from left to right) sugar beet.

growing and became considerably longer than in the controls. This unduly prolonged growth in length is probably caused by the total lack of seed setting of the haploid. The top shoot of the haploid gradually became fasciated.

As shown in Fig. 2, the floral parts of the haploid were decidedly smaller and more delicate than in the diploid. The triploids and tetraploids had, as usual, larger flowers than the diploids. The flowers of the haploid were often somewhat malformed; the pictured flower, for instance, had 6 instead of the normal 5 petals and stamina.

The pollen fertility was determined on various occasions. The haploid showed at early stages a surprisingly high pollen fertility, but later on the anthers were often quite empty. On one occasion the percentage of filled pollen in the four ploids was: 92,3, 98,0, 86,3 and 93,0. The haploid thus showed higher pollen fertility than the triploid and about the same fertility as the tetraploid. This means that the pollen tetrads, in spite of the irregularities of meiosis, may develop rather regularly. The pollen grains may survive for some time as free pollen grains before degenerating, although most of them must be deficient. Measurements of pollen diameters gave an equally rising curve for diploids, triploids and tetraploids. The haploid lies only slightly below the diploid. The means of 100 cells were 18,8, 19,5, 22,3 and 24,8 μ for haploid, diploid, triploid and tetraploid respectively. As already mentioned, the haploid was absolutely seed-sterile, although it was repeatedly dusted over with pollen from the diploid sister plant.

II. CYTOLOGY.

The haploid sugar beet showed rather clear and favourable chromosome conditions in the root tips (Fig. 3 *a*, *b*). Especially in side views of anaphase it was possible to trace the position of the centromeres. Their position was intercalary in all chromosomes. Eight of the chromosomes were attached medially-submedially, the ninth chromosome was somewhat more asymmetrical. This latter one is most probably the satellite chromosome. As already known, certain size differences occur within the beet idiogram. The largest chromosome is 2,5—2,8 μ in length, the smallest is 1,5—1,7 μ. Between these extremities the transition is continuous. Satellites were not seen regularly, but especially in one fixation (Fig. 3 *a*) there often occurred terminal differentiations, probably of the same kind as those observed in haploid rye (LEVAN, 1942 a).

A typical pachytene stage occurs in the beet haploid as well as in *Antirrhinum* (ERNST, 1939) and in the rye haploids. Although this

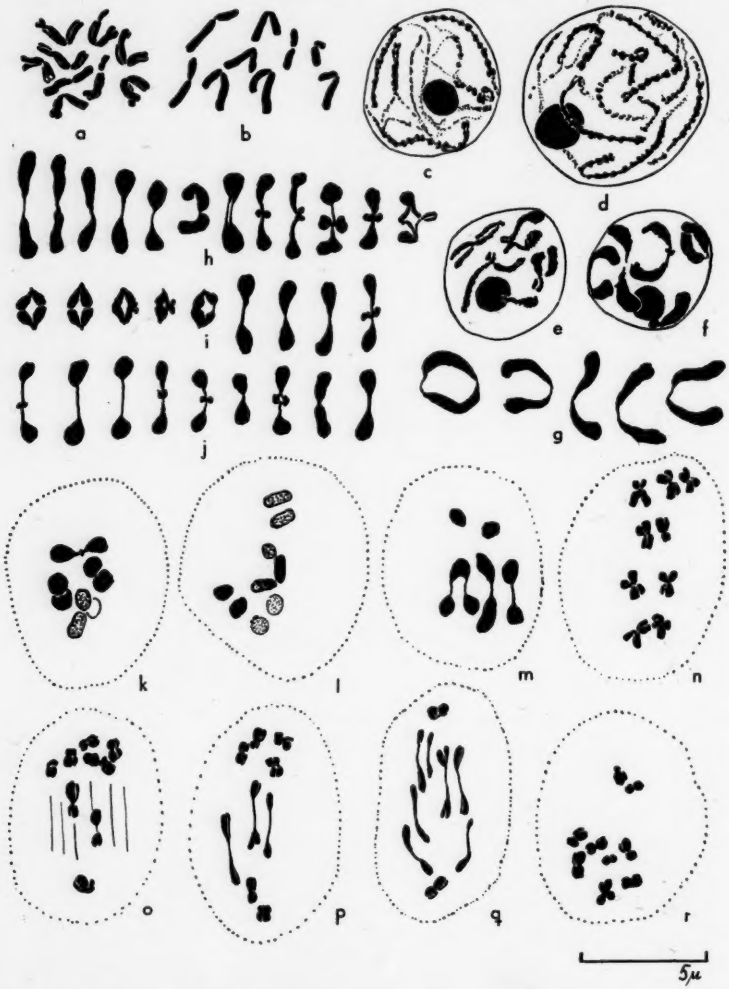

Fig. 3. *d*, *i* and *j* diploid, the rest haploid sugar beet. *a*—*b*: root mitoses; *a*: metaphase in polar view, *b*: an analysed anaphase group in side view. *c*—*r*: meiosis; *c*—*d*: pachytene, *e*: early diakinesis, *f*: later diakinesis, *g*: chromosome pairs at diakinesis, *h*: instances of metaphase I bivalents in the haploid, *i*, *j*: two analysed metaphase I plates of the diploid, *k*: metakinesis, *l*, *m*: metaphase I, *n*—*q*: anaphase I, *r*: metaphase II. — × 3900.

stage is very much less clear in *Beta*, it was with certainty seen that paired segments occurred regularly in the pachytene nuclei (Fig. 3 *c*). All transitions were found between total lack of pachytene pairing and almost complete pairing. More often than in rye with its longer chromosomes, paired chromosome ends were seen in the beet. This condition, that the chromosome ends pair at the same level even if the interior parts of the chromosomes remain unpaired, may be taken as a sign that the zygotene pairing in *Beta* is of the proterminal type.

On a few occasions one or two cells of a pollen chamber at pachytene were found to be of a larger size and evidently to have the diploid chromosome number (Fig. 3 *d*). They were surrounded by haploid cells at the same stage. This condition permitted a good control of the fact that such chromosome threads as in haploid cells were interpreted as paired really were paired. In the diploid cells pairing at this stage seemed to be normal although not complete. Solitary diploid cells met with at later stages showed that chiasmata were not formed regularly within them. One diploid cell at metaphase I, for instance, had only one bivalent and 16 univalents, these latter arranged in pairs. Nuclear and nucleolar size in haploid and diploid cells at pachytene is seen from Fig. 3 *c* and *d*.

In *Beta* it is impossible to follow the prophase step by step as in *Secale*. At diplotene, for instance, the chromosomes are so weakly stained that I have been unable to observe any details. At early diakinesis (Fig. 3 *e*) the chromosomes frequently occur in pairs, their point of contact often being intercalary. This pairing is most often purely spiralization pairing or residuary pairing (LEVAN, 1942 a), but at times real interstitial chiasmata were met with. At later diakinesis (Fig. 3 *f*, *g*) the chromosomes are shorter and it is often difficult to trace their residuary pairing. The contact points of the pairs is now almost always terminal.

After the disappearance of the nuclear membrane, the chromosomes are gathered towards the centre of the cell. The arrangement in pairs may sometimes still be quite evident (Fig. 3 *k*). The chromosomes now react towards the spindle and the difference between chiasma pairing and residuary pairing becomes evident. While chromosomes held together by chiasmata form bivalents, or in solitary cases trivalents, which are co-orientated on the spindle, the univalents are scattered out irregularly (Fig. 3 *l*, *m*).

Real chiasmata occurred rather often in the haploid at metaphase I. Their frequency varied somewhat in different slides. In two slides,

which were especially well-fixed, the following frequency of different elements were recorded:

Configuration	Number of cells	Percentage
9 I	161	56,1
7 I + 1 II	97	33,8
5 I + 2 II	21	7,3
3 I + 3 II	2	0,7
4 I + 1 I + 1 III	2	0,7
6 I + 1 III	3	1,1
2 I + 2 II + 1 III	1	0,4

Thus, in these slides there occurred one chiasma in about every second cell. This frequency is probably among the highest present in this haploid. In other fixations the chiasma frequency was estimated at 0,3—0,1 per cell.

Fig. 3 *l* and *m* represent two extremes in chiasma occurrence, in *l* all chromosomes are univalents and in *m* one trivalent and two bivalents are present. Bivalents from the haploid are pictured in Fig. 3 *h* and bivalents from a diploid sister plant are shown in Fig. 3 *i* and *j*. The bivalents in the haploid are always rods. The chiasmata are usually terminal, but not infrequently interstitial chiasmata also occur at metaphase I. Most chiasmata are symmetrical, which indicates that the pachytene pairing has started at the ends or, in any case, at the same distance from the ends in both chromosomes involved. Exceptionally, however, clearly asymmetrical chiasmata were also found (e. g. the bivalent last in the row of Fig. 3 *h*). Unfortunately, the size differences between the chromosomes are not considerable enough to allow of the certain identification at metaphase I of any definite chromosome. As a rule, no pronounced size difference between the two paired chromosomes of a bivalent was seen, which probably would have been the case if the largest and the smallest chromosome were involved in the same bivalent. This suggests that the pachytene pairing is not absolutely at random. On the other hand, rather evident size differences occurred between different bivalents, which goes to show that it cannot be the same two chromosomes which preferably paired.

Generally speaking, the bivalent shape of the haploid is in agreement with that of the diploid sugar beet. This may be seen by comparing Fig. 3 *h* with *i* and *j*, in which two metaphase plates of a diploid sister plant have been analysed. The rod bivalents are of exactly the same type. In the diploid ring bivalents with two chiasmata also occur.

Thus, the cell in Fig. 3 *i* has no fewer than 5 rings, a maximal number encountered in the plant studied. In 25 analysed cells of the diploid the frequency of rods and rings was as follows:

Rods	Rings	Number of cells
9	0	6
8	1	11
7	2	5
6	3	2
5	4	0
4	5	1

The number of chiasmata per cell varied from 9 to 14 (mean 10,3), which is about 20 times more than in the haploid. The number of non-terminalized chiasmata of the diploid was 0 to 6 (mean 2,6).

Anaphase I of the haploid shows some variation in type. In some cases all chromosomes assume anaphase appearance (Fig. 3 *n*), the split between the chromatids being clearly visible. The univalents are then assorted to the poles, each univalent proceeding to the nearest pole. Bivalents are stretched out between the poles. The chromosomes of the bivalents separate eventually and go one to each pole. This is the normal type of anaphase I. In certain pollen chambers, however, several (1—8) univalents quite regularly went to the equator and remained there exhibiting all signs of being about to divide (Fig. 3 *o*—*q*). They were very much extended, indicating that their centromeres were incapable of dividing. Their centromeric regions were drawn out into long bridges, sometimes reaching from pole to pole. Pictures such as Fig. 3 *p* and *q* strongly suggest that misdivision may take place. In the rye haploid similar pictures were met with, and there it could be actually demonstrated in metaphase II that misdivision at anaphase I was of frequent occurrence.

The second division of the haploid sugar beet was often rather irregular, showing vagabonding chromosomes, supernumerary plates, etc. In one slide the following number of elements were counted in the different metaphase plates; the detailed study of this stage was impeded by the unfavourable condition of the material.

Number of chromosomes in the plates	9	8	7	6	5	6	5	4	8	10	12
	0	1	2	3	4	2	3	4	2	2	0
	—	—	—	—	—	1	1	1	—	—	—
Number of cases	4	4	3	2	4	2	2	1	1	1	1

The last three configurations must contain chromosomes which have undergone division at the first anaphase.

III. CONCLUSIONS.

Having in my above-mentioned study of haploid rye discussed at some length the mechanism of meiosis in this haploid and also made comparisons with a number of other haploids studied up to that time, I need now only very briefly state that the sugar beet haploid shows agreement with the rye haploid on many points which were shown to be characteristic of the rye haploid and presumed also to occur in most other haploids.

Thus, a quite striking feature is that the beet haploid, as well as all haploids previously studied, strives to undergo the typical meiotic course with all that this involves in regard to very characteristic developmental details. At a certain stage in the life history of the plant the meiotic mechanism starts in a haploid as in all other ploids. The mechanism of meiosis is undoubtedly adapted for the diploid chromosome number. In those haploids which occasionally occur in diploid species meiosis resembles as closely as possible the conditions in the corresponding diploids. Owing to the absence of homologous chromosome pairs the entire mechanism, however, ends in failure. On the other hand, such haploids as are normally present in the life cycle of certain organisms have developed some deviation in their meiotic course to enable them to escape the meiotic breakdown inevitable in the casually originating haploids.

It is rather suggestive that a typical pachytene stage is found in the three haploids which have so far been studied in detail. The pachytene stage consequently does not necessarily require the presence of two homologous genomes, within which every chromomere has a homologous partner to become paired with. Pairing occurs in the haploid although only one chromosome of each kind is present. It is reasonable to believe that the main course of pairing is genetically fixed in the species, the haploid trying to imitate the diploid as closely as possible. In the diploid rye and sugar beet the chiasmata are situated at or close to the chromosome ends, probably due to pachytene pairing starting from the ends. The same general picture is characteristic of the haploids of these species. The pachytene pairing seems to be proterminal and the bivalent shape at metaphase I agrees with that in the diploids.

The great difference between meiosis in haploids and corresponding diploids is, above all, that the haploid pairing generally gives rise only to very few chiasmata. Consequently, when the chromosomes contract during diplotene-diakinesis, the haploid pairing gradually disappears. It is maintained sometimes through the entire prophase as a spiralization pairing that is of a similar nature to the pairing found in some asynaptic plants (e. g. LEVAN, 1940), but at metaphase I, if not earlier, it is lost.

During the study of the rye haploid I attempted a statistical analysis of the few real chiasmata, which were found at metaphase I in haploids. I was able to demonstrate that all chiasmata (in *Oenothera*) or part of them (in *Antirrhinum* and *Secale*) were distributed according to a POISSON series, i. e. the chiasmata may have been caused by purely chance factors. This condition may be taken as an indication that chiasmata in haploids are not formed between certain homologous segments but are scattered out at random over the entire paired chromosome length.

This condition has also been tested in the beet haploid, and here, too, the agreement between the chiasma frequency found and POISSON was rather close. From the table on page 406 the following values are obtained:

Number of chiasmata per cell:	0	1	2	3	4	5
» » cells:	161	97	24	4	1	0
Expected according to POISSON: ..	163,8	91,9	25,8	4,8	0,7	0,1

$\Sigma\chi^2 = 0{,}5248$. Thus, the cytological analysis of the sugar beet haploid gives support to the hypothesis earlier suggested, viz. that the chromosome pairing in haploids depends on purely chromosome-mechanical factors and that as a rule the chiasmata formed should not be taken as proof of homologies within the genome. This hypothesis thus implies a warning that conclusions concerning the evolution of the genome by duplications should not be drawn merely from observations of bivalents during metaphase I of haploids.

SUMMARY.

The morphology and cytology are described in a haploid plant of sugar beet that occurred in the progeny of a colchicine-treated diploid. The haploid had a typical pachytene stage with, sometimes, rather complete chromosome pairing. During later meiotic prophase pairing was

of two kinds, spiralization (residuary) pairing and chiasma pairing. The former type disappeared before metaphase I. The chiasma frequency was shown to agree with a POISSON distribution. The possible bearing of this on the understanding of the nature of the chiasmata is pointed out.

LITERATURE CITED.

1. ERNST, H. 1939. Zytogenetische Untersuchungen an haploiden Pflanzen von *Antirrhinum majus* L. — Zschr. f. Bot. 35: 161—190.
2. LEVAN, A. 1940. The cytology of *Allium amplectens* and the occurrence in nature of its asynapsis. — Hereditas XXIV: 353—394.
3. — 1942 a. Studies on the meiotic mechanism of haploid rye. — Hereditas XXVIII: 177—211.
4. — 1942 b. The effect of chromosomal variation in sugar beets. — Hereditas XXVIII: 345—399.

THE TRIPLOID PROGENY OF THE CROSS DIPLOID × TETRAPLOID POPULUS TREMULA

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I. INTRODUCTION.

THE first spontaneous, triploid aspen — a male clone — was discovered by NILSSON-EHLE on the Bosjökloster estate in Scania in 1935 (NILSSON-EHLE, 1936). The cytological behaviour of this aspen was studied by MÜNTZING (1936), who found that unreduced pollen-grains were developed in some frequency. MÜNTZING also pointed out the possibility this implied of producing tetraploid individuals by crossing the triploid with diploid female trees.

Under NILSSON-EHLE's guidance a number of such crosses were carried out in the winter of 1937. One tetraploid plant appeared among the progeny obtained (NILSSON-EHLE, 1938; BERGSTRÖM, 1940). Its mother was a diploid aspen from Sätra Bruk in Västergötland.

The tetraploid plant was propagated by grafts and root slips in the spring of 1938 by Mr. H. JENSEN, Ramlösa. In the spring of 1944 some of these clonal individuals flowered for the first time, it being then found that the tetraploid was a male. It was now possible to backcross the tetraploid with a diploid aspen, this work being carried out at the Institute for Forest Tree Breeding, Ekebo, Källstorp. The chief purpose of these crosses was to realize the plan NILSSON-EHLE (1938) had put forward for the mass production of triploid seed-plants for silviculture. During the summer of 1944 about 30.000 such plants were raised. In addition, some investigations were undertaken to ascertain the differences existing between the diploid and the triploid aspen. The first results of these studies are presented below.

II. MATERIAL AND METHODS.

The material comprises five progenies from the crosses between the tetraploid aspen as father and various diploid trees as mothers as well as five progenies of crosses between the same mothers and diploid

trees as fathers. The crossings were carried out and the material cultivated in the manner described by JOHNSSON (1942).

The following combinations were included:

Female parents	Male parents	Cross No.
Populus tremuloides	The tetraploid	44— 1
	Hamra No. 10, Östergötland..	44— 2
Sätra No. 4, Västergötland	The tetraploid	44— 7
	Sätra No. 1, Västergötland ..	44— 8
Häggeby, Uppland	The tetraploid	44— 9
	Hamra No. 10	44—10
Skeppmora, Västmanland	The tetraploid	44—11
	Sätra No. 1	44—12
Ås No. 5, Akershus, Norway	The tetraploid	44—25
	Ås No. 20, Akershus, Norway	44—26

During the summer these ten crosses were planted in cold frames as comparative trials on the principle of FISHER's block method. Each patch consisted of a frame-sash covering 112 plants, the replications being three in number. The special methods of examination employed will be described in the course of the following account. The results are evaluated with guidance of the *z*-test, according to FISHER (1938). Probabilities $\leqq 0{,}001$ are denoted with $^{+++}$, between 0,01 and 0,001 with $^{++}$ and between 0,05 and 0,01 with $^{+}$.

III. FERTILITY OF THE TETRAPLOID.

The tetraploid proved to be a pure male aspen, without any trace of intersexuality. During flowering it behaved perfectly normally. The floral organs were well developed and larger than in most aspens, though not conspicuously large. Pollen was liberated in large quantities. The pollen-grains were very equal in size, fully as uniform as those of diploid aspens, contrasting in this respect sharply with the varying pollen of the triploids, as is evident from the following data.

	Diameter of pollen-grains in units											
Tree	10	11	12	13	14	15	16	17	18	19	20	Mean
The tetraploid				5	28	36	20	11				$15{,}04 \pm 0{,}11$
Gräfsnäs, triploid	2	11	25	37	17	8	3	3	4	6	2	$13{,}47 \pm 0{,}21$
Hamra No. 10, diploid	3	10	42	33	12							$12{,}41 \pm 0{,}09$

There is a continuous rise in the mean diameter of the pollen-grains from the diploid to the tetraploid state. In the cases set forth

here the triploid has, on an average, a pollen diameter that is 8,5 % larger than that possessed by the diploid, and the pollen diameter of the tetraploid exceeds that of the triploid by 11,7 %.

The seed-setting after pollination with pollen from the tetraploid was a little lower than that after application of pollen from the diploids. The number of seeds per capsule was determined for four tetraploid and four diploid crosses. The figures given indicate the mean numbers for ten capsules.

Mother tree	*P. trem.*		Sätra		Häggeby		Skeppm.		Mean	
Father tree	4*x*	2*x*	4*x*	2*x*	4*x*	2*x*	4*x*	2*x*	4*x*	2*x*
No. of seeds per capsule	5,0	6,9	8,3	9,7	7,1	9,2	7,3	8,8	6,9	8,7

Thus, the diminution in seed production after pollination with pollen from the tetraploid amounts to 21 %. This shows a not inconsiderable zygote and embryo lethality. The reduction in fertility, however, is not so great as after pollination with pollen from the triploid aspen, in which case JOHNSSON (1940) found 9,9 seeds per capsule after diploid pollen and only 5,1 after triploid.

IV. CHROMOSOME NUMBER OF TETRAPLOID PROGENY.

Chromosome counts were made on altogether 160 plants obtained from four crosses. The results were as follows:

Cross	Chromosome number														
No.	47	48	49	50	51	52	53	54	55	56	57	58	59	*n*	Mean
44— 1						2	2	3	6	8	24			45	55,96
44— 9	1	1	3	3	3	1	1	5	8	7	21	2	1	57	54,95
44—11				2	1			4	3	4	19	3		36	55,92
44—13					1			2	2	3	10	3	1	22	55,96
Total	1	1	3	5	5	3	3	14	19	22	74	8	2	160	55,57

The triploid number, $2n = 57$, was found in 74 plants of 160 or in 46,3 % of the progeny. As many as 86,9 % of the progeny fall within the range 54—59 chromosomes. A lower number than 57 is shown by 76 plants, i. e. 47,5 % of all plants, while only 10 plants, corresponding to 6,3 % of all, have a higher chromosome number than 57.

Some uncertainty in the determinations — see JOHNSSON, 1940 — may have contributed to the dispersion in chromosome numbers. None the less, the result is undoubtedly significant. Thus, it may be affirmed that on being crossed with diploids the tetraploid gives mainly triploid

offspring. However, especially small deviations from the triploid number are common, in particular aberrant individuals with lower numbers than the triploid. The meiosis of the tetraploid must consequently be rather irregular, since the progeny have varying chromosome numbers and the seed-setting after pollination with the tetraploid shows a diminution. In all probability this reduction is due to the fact that gametes emanating from the tetraploid have a lethal effect. Probably a greater or less portion of this gamete fraction is numerically aberrant. The meiotic irregularities of the tetraploid must lead to chromosome elimination, as the minus variates around the triploid number are far more numerous than the plus and the average chromosome number of the progeny is lower than 57. Direct observations of the tetraploid's meiosis could not be made, as the specimens prepared were unsuitable for this purpose.

This meiotic instability, which is clearly apparent in the tetraploid aspen, also recurred in practically all the newly produced autotetraploids.

V. HABITUAL APPEARANCE OF THE TETRAPLOID PROGENY.

1. *Leaf-size.* — The triploid *Populus* progenies differ markedly from the diploid offspring. This difference is chiefly marked by the larger leaves of the triploids. Measurements of the leaf-sizes are submitted in Table 1. The size of the leaves has been worked out by weighing carboard figures cut out to correspond to the leaves. Every figure recorded in the table is the mean of two average leaves taken in the middle of the plants.

Analysis of the variance showed that the predominant cause of the variation is the difference in chromosome number. The variance due to this difference compared with that due to error gives $z = 2{,}3179^{+++}$.

Within varieties having the same chromosome number there are also significant differences in leaf-size. As a measure of significance for diploid varieties $z = 1{,}0110^{+++}$ is obtained, and for triploid varieties $z = 0{,}7591$, which almost amounts to $P = 0{,}01$, the latter corresponding actually to $z = 0{,}7607$. The difference demonstrated here refers exclusively to the two *Populus tremuloides* progenies, which have considerably larger leaves than the pure *P. tremula* offspring.

2. *Leaf-thickness.* — The leaves of the triploids differ from those of the diploids not only respecting area of leaf-surface but also in

TABLE 1. *Size of the leaves, cm².*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	108,1	74,8	79,9	47,9	87,9	51,9	85,8	45,3	76,0	52,4	87,5	54,5
Block II ...	132,1	89,3	88,3	58,5	93,1	55,2	114,7	51,6	93,5	53,4	104,3	61,6
Block III...	108,0	88,5	96,7	55,6	81,4	84,1	83,0	44,0	87,1	56,6	91,2	65,8
Mean.....	116,3	84,2	88,3	54,0	87,5	63,7	94,5	47,0	85,5	54,1	94,4	60,6

TABLE 2. *Thickness of the leaves, 1 unit = 0,01 mm.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	22,4	19,2	23,3	21,2	23,7	21,1	25,0	20,2	22,9	19,0	23,5	20,1
Block II ...	19,9	18,4	23,1	21,9	22,0	19,3	22,9	19,9	21,1	19,4	21,8	19,8
Block III...	22,5	18,9	23,9	21,3	23,0	20,5	22,5	20,7	22,8	19,8	22,9	20,3
Mean.....	21,6	18,8	23,4	21,5	22,9	20,3	23,5	20,3	22,3	19,4	22,7	20,0

TABLE 3. *Lengths of stomata, 1 unit = 2,4 μ.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	15,3	11,8	15,0	10,5	14,8	12,4	14,3	10,8	15,1	13,1	14,9	11,7
Block II ...	14,3	12,4	16,1	12,3	14,5	11,3	15,1	12,5	15,7	11,8	15,1	12,1
Block III...	13,1	10,9	14,1	9,7	13,4	12,9	14,5	12,1	13,8	9,4	13,8	11,0
Mean.....	14,2	11,7	15,1	10,8	14,2	12,2	14,6	11,8	14,9	11,4	14,6	11,6

having distinctly greater thickness, in addition to which they are a darker green.

Measurements were made of the thickness of the leaves, taken in the angle of the midrib with its first lateral branch. They were made with a cover-glass tester on ten leaves from as many different plants out of each patch. Table 2 presents the results obtained.

The analysis of the variance gave as measures of significance $z = 2{,}6865^{+++}$ for the chromosome number difference, $z = 1{,}3273^{+++}$ for differences between diploid varieties, and $z = 1{,}1474^{+++}$ for differences between triploid varieties.

With regard to the varietal differences within groups possessing the same chromosome number, the thin leaves of the *P. tremuloides* cross-products are especially conspicuous. The Ås offspring also have thin leaves, while the Sätra, Häggeby and Skeppmora offspring correspond closely with one another and have thicker leaves, the Sätra having the highest values. Thus, although the most important cause of the variation in leaf-thickness is the difference in chromosome number, there also occurs here a significant variation of another kind.

3. *Other habitual characters.* — The most characteristic differences between the triploid and diploid progenies have reference to the leaves. The structure of the stem, however, likewise exhibits differences. Compared with their height the triploids possessed a somewhat thicker stem — see below — and hence gave a more robust impression.

VI. SIZE AND NUMBER OF CELLS.

1. *Length of stomata.* — The stomata appear to be always larger in autopolyploids than in the original diploid forms. Such is also the case in *P. tremula* (MÜNTZING, 1936; BERGSTRÖM, 1940; JOHNSSON, 1940). The stomatal lengths found in the material under investigation here are collected in Table 3. The patch values recorded are means of 50 stomata.

The difference between diploids and triploids is beyond all doubt, with $z = 2{,}2185^{+++}$. No significant variation appears, however, between varieties with the same chromosome number.

Thus, the stomata in the triploids were 26 % longer than those in the diploids. If the breadth of the stomatal cells follows the same line and the other cells follow the stomatal cells in this respect, the ratio between the cellular surfaces in triploid and diploid aspens will be $1{,}59 : 1{,}00$. Therefore, if the number of cells in different organs is the

same in both chromosome-number races, the surfaces of the organs will be in the ratio $1{,}59 : 1{,}00$. For leaf-surfaces $1{,}56 : 1{,}00$ was obtained, as seen in Table 1.

2. *Number of stomata per unit surface.* — The larger the cells, the smaller must their number be per unit surface. This relation ought to find expression in the number of stomata per unit of surface, provided the number of cells in the tissues lying between the stomata is the same. To investigate this point the number of stomata per unit surface was determined. This was done with the aid of net-micrometer, object glass 40 and eyepiece 7. The results are furnished in Table 4, in which the patch data are means of 10 surface units.

The only characteristic cause of variation is the difference in chromosome number with $z = 1{,}9336^{+++}$. The number of stomata per unit surface in triploids and diploids shows the ratio $1{,}00 : 1{,}53$, which is in good agreement with the corresponding calculated ratios for cell-size, $1{,}59 : 1{,}00$, and for leaf-area, $1{,}56 : 1{,}00$. For the leaves, thus, it was possible to determine that the number of cells in their superfices is the same in diploids and triploids, and that the increase in the area of the leaves is directly proportional to the increase in the surface of the cells. From this it follows that the number of stomata per leaf should be the same both in diploids and triploids. Counts gave 81.861 stomata per leaf in the diploids and 83.901 in the triploids, i. e. a difference amounting to only 2 per cent.

3. *Length of wood cells.* — To investigate the influence of the chromosome number on cell-size in other organs than the leaves measurements were taken of the wood cells in the stem. For this purpose three plants of medium size were employed from each patch. Test-pieces about 2 cm. long were taken from the middle of the stem. These were mascerated by boiling for two hours in alcoholic nitric acid according to KÜRSCHNER's technique, and were then beaten up in water. When the specimens were being prepared glycerine was added. The preparations were projected with the microscope, objective $\times 5$ and eyepiece $\times 7$. The projections of the wood cells were measured with dividers and millimetre scale. Measurements were taken of the lengths of 100 wood cells from each plant, and the patch values given in Table 5 are the means of 3×100 cells.

The only outstanding source of variation here is the difference in chromosome number. The reliability of the difference in wood-cell length between diploids and triploids is indicated by $z = 1{,}9901^{+++}$. Hence the increase in chromosome number has brought about a size-

TABLE 4. *Number of stomata per leaf-surface, 1 unit = 0,7921 mm².*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	6,7	10,4	6,6	11,6	7,9	9,8	7,0	11,4	7,9	8,5	7,2	10,3
Block II ...	6,6	6,8	6,5	10,6	6,9	11,8	6,4	10,9	6,9	12,3	6,7	10,5
Block III...	6,3	9,9	6,8	12,5	9,0	9,7	6,0	8,9	8,1	15,1	7,2	11,2
Mean..... ...	6,5	9,0	6,6	11,6	7,9	10,5	6,5	10,4	7,6	12,1	7,0	10,7

TABLE 5. *Lengths of the wood cells, 1 unit = 0,01205 mm.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	41,9	36,1	44,4	39,6	45,8	38,5	44,6	36,7	44,1	38,5	44,2	37,9
Block II ...	43,5	41,7	46,8	37,7	49,3	40,6	43,4	40,0	49,7	39,4	46,5	39,9
Block III...	47,6	38,3	48,4	42,3	51,0	44,0	48,5	40,2	46,9	41,0	48,5	41,2
Mean.....	44,3	38,7	46,5	39,9	48,7	41,0	45,5	39,0	46,9	39,6	46,4	39,6

TABLE 6. *Diameters of the wood cells, 1 unit = 2,4 μ.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	10,9	10,4	10,2	8,9	9,5	9,1	10,5	8,5	11,0	7,9	10,4	9,0
Block II ...	12,4	10,9	11,5	8,0	10,5	10,1	9,9	9,2	11,5	8,6	11,2	9,4
Block III...	9,3	8,1	11,8	8,4	11,3	10,9	11,5	9,5	12,5	9,0	11,3	9,2
Mean.....	10,9	9,8	11,2	8,4	10,4	10,0	10,6	9,1	11,7	8,5	11,0	9,2

increase not only in the cellular components of the leaves but also in those of the wood. The average increase in the length of the wood cells amounts to 17 % as against 26 % for the stomatal cells. Whether this measured difference corresponds to a real effectual difference cannot be immediately decided.

4. *Diameter of wood cells.* — The diameter of the wood cells was determined by direct measurement with the eyepiece micrometer, 50 cells being measured from each specimen employed for the length measurements. Hence the values in Table 6 are means of 150 cells, 50 from each of three plants.

No differences have made their appearance within varieties having the same chromosome number, while difference in chromosome number has brought about a difference in cell-diameter, the statistical reliability of which is indicated by $z = 1{,}6460^{+++}$. According to the measuring results, the triploids have 20 % wider cells than the diploids. Expressed in absolute units the triploids have a wood-cell volume of $k_t \,.\, 3{,}20 \times 10^{-4}$ c.mm. and the diploids one of $k_d \,.\, 1{,}84 \times 10^{-4}$ c.mm. The factor k is an expression for deviations of the cells from the cylinder having the measured cell-length and cell-diameter. This deviation is mainly due to the narrowing of the cells towards their ends. This constriction should be fairly equal for both triploids and diploids, and therefore k_t may be approximately taken as equal to k_d. The triploid/diploid ratio in respect of wood-cell volume would therefore be $3{,}20 : 1{,}84$ or $1{,}74 : 1{,}00$. If this applies to all the cellular elements in the stem, then the growth of the stem per period of vegetation in triploids and diploids will likewise show the ratio $1{,}74 : 1{,}00$, provided the rate of cell division in the cambium is the same for both chromosome-number groups and the divisions go on during equally long periods.

VII. GROWTH OF STEM.

1. *Growth in length.* — The patch means of the plant-height at the end of the growth period in the autumn are given in Table 7. The patch values are means of all the plants in the patches, 112, or slightly under owing to one or two plants having died or been damaged during the summer.

Here, the difference in chromosome number is no longer, as previously, any major cause of variation; on the contrary, it is not responsible for any general difference at all. The measure of significance, z, has the value $0{,}3702$. There makes its appearance instead a

TABLE 7. *Plant heights, in the autumn, cm.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	63,1	91,9	43,1	45,3	46,3	54,0	45,6	37,5	50,0	28,9	49,6	51,5
Block II ...	81,7	88,9	47,4	56,3	55,1	67,3	44,5	41,6	75,9	55,9	60,9	62,0
Block III...	76,5	91,6	50,4	55,8	61,2	79,9	46,9	52,4	70,3	62,4	61,1	68,4
Mean.....	73,8	90,8	47,0	52,5	54,2	67,1	45,7	43,8	65,4	49,1	57,2	60,7

TABLE 8. *Stem diameters, in the autumn, mm.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	4,8	6,4	3,7	4,0	4,1	4,1	4,2	3,4	4,1	3,1	4,2	4,2
Block II ...	5,6	6,0	4,1	4,2	4,3	4,6	4,3	4,2	5,2	4,2	4,7	4,6
Block III...	5,2	6,3	4,4	4,2	4,6	5,3	4,2	3,9	4,8	4,8	4,8	4,9
Mean.....	5,2	6,2	4,1	4,2	4,3	4,7	4,2	3,8	4,7	4,0	4,5	4,6

TABLE 9. *Stem volume, cm^3.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	3,63k	9,41k	1,48k	1,81k	1,95k	2,27k	2,01k	1,08k	2,10k	0,69k	2,24k	3,05k
Block II ...	6,40k	8,00k	2,19k	2,48k	2,55k	3,56k	2,06k	1,83k	5,13k	2,47k	3,67k	3,67k
Block III...	5,17k	9,09k	2,44k	2,70k	3,24k	5,61k	2,07k	1,99k	4,05k	3,59k	3,39k	4,60k
Mean.....	5,06k	8,83k	2,04k	2,33k	2,58k	3,81k	2,05k	1,63k	3,76k	2,25k	3,10k	3,77k

significant variation within the chromosome-number races. For the variation within the triploid crosses z is $1{,}1701^{+++}$, for that within the diploid it is $1{,}6157^{+++}$. Within diploids as well as triploids the *P. tremuloides* cross is much above the pure *P. tremula* crosses. Thus, we are confronted here with a pronounced heterosis effect. A contributory cause of this heterosis may be, and probably is, that the maternal parent of *P. tremuloides* was derived from a more southern latitude than the place of trial. As a matter of fact, it has been found that transplantation northwards of, e. g., *P. tremula* results in increased growth up to the limit af hardiness, *photoperiodicity* (SYLVÉN, 1940). The source of the *P. tremuloides* tree employed in the trials is certainly unknown, but, as the greater part of the area of the species is south of 56° — the latitude of the place of cultivation, be it noted —, it is very probable that the tree belongs to a southern race. On the other hand, all parents of the *P. tremula* included in the trial originate from more northern regions than the place of trial.

The standard deviation of the error amounts to 6,52, which makes the standard error of the difference between varieties 5,32 cm. Thus, among the pure diploid aspen crosses, Häggeby × Hamra is characterized by a significantly greater plant-height than the three others, which do not exhibit any sure difference among themselves. Of the pure triploid aspen crosses, Ås × the tetraploid is definitely higher than the other three. Verified differences between diploid and triploid half-sibling families exist in two cases. In one — that with *P. tremuloides* as mother — the diploid family is superior, whereas in the other — that with Ås as mother — the triploid family occupies that position.

2. *Growth in diameter.* — The stem-diameter was measured 10 cm. above the ground on all plants in the patches, and the means for the respective patches are set out in Table 8.

Here, too, the difference in chromosome number does not prove to be any cause of variation. Instead, a significant variance appears within both chromosome-number groups, represented by $z = 1{,}6185^{+++}$ and $z = 0{,}9983^{++}$ for diploids and triploids respectively. This variation is entirely due to the two *P. tremuloides* crosses, which undoubtedly have a greater diameter than the pure aspen crosses.

Between growth in height and growth in diameter there is a very strong positive correlation with $r = 0{,}958$. The measure of significance for this correlation is $t = 17{,}676$, which gives an exceedingly small probability for randomness.

3. *Growth in volume.* — The best measure of a stem's growth is

manifestly its cubic content. If the height (h) and the diameter (d) of the stem are measured at a definite point, the volume of the stem may be expressed as $k \cdot \left(\frac{d}{2}\right)^2 h$, where the constant k includes π as well as a factor that represents the deviation of the stem from the cylinder of h height and d diameter. This constant may be assumed as approximately equal for all the varieties, and hence the computations of stem-volume recorded in Table 9 will no doubt give a good view of the relative growth in volume during the first summer.

Quite the greater part of the variance is intra-group variance with $z = 2{,}5281^{+++}$ for triploid varieties and $z = 1{,}0225^{+++}$ for diploid. On the other hand, the difference in chromosome number has not manifested itself in any definite difference in volume growth; z is $0{,}8199^{+}$. The intra-group variance is based almost entirely on the differences between the *P. tremuloides* crosses and the pure *P. tremula* crosses. With a standard error per patch of $0{,}6611\,k$, the standard error per varietal difference of $0{,}66\,k$ is obtained. A significant difference in stem volume must therefore amount to about 1,98 c.c. From this it is evident that with the diploid crosses the family having *P. tremuloides* as mother is superior to all the other families. Among the pure *P. tremula* crosses the only decided difference appears between Häggeby × Hamra and Skeppmora × Sätra, showing superiority for the former. Within the triploid families the *P. tremuloides* family is likewise distinctly superior, though the difference between *P. tremuloides* × the tetraploid and Ås × the tetraploid is not statistically verified. The only case in which half-sibling diploid and triploid families clearly differ is the *P. tremuloides* families, with the diploid family as the superior.

In the triploids the volume of the wood-cells proved to be 74 % greater than in the diploids. However, there is no increase in volume growth corresponding to this in the triploids. Growth started under artificial conditions simultaneously for both chromosome-number races. It terminated under the influence of natural climatic conditions. No difference whatever between the chromosome-number races in the rate at which growth declined or its definite cessation in the autumn could be observed. It is therefore manifest that the rate of cell-division in the cambium and the vegetation point of the top must be so much lower in the triploids that this lower speed of cell-division counteracts the greater cell volume. This point is dealt with more closely later on in this paper, and the significance of the result at a high age is also discussed.

VIII. PHYSIOLOGICAL INVESTIGATIONS.

1. *Osmotic pressure.* — Determination of the plasmolysis was performed in the epidermis of the petiole, a tissue that is well suited for such investigations on account of the regular, rectangular shape of the cells. There was however a somewhat disturbing element, viz. some of the cells were anthocyan-bearing, others not. The plasmolysis was determined in cane sugar solutions with intervals of 0,05 mol/lit. A series of determinations were undertaken on each patch, and the results have been drawn up in Table 10. From the table it is immediately seen that no differences of significant value appeared in osmotic pressure.

2. *Transpiration intensity.* — The transpiration intensity was estimated on severed leaves, one leaf of medium size being taken from each patch. Ten leaves from each block were included in the same series. The test was carried out in the laboratory during the period July 9—11 under a temperature of about 29° C and lasted three hours. Table 11 shows the computed exhalation of water per 50 cm^2 of leaf-surface per hour. The mean for the whole test shows in all cases a higher transpiration for the diploid families, but the results for the patches are not in full agreement. The difference between the chromosome-number races is likewise not statistically demonstrated.

z for the difference between the chromosome-number races amounts only to 0,4338, corresponding to $P > 0{,}10$. Nor is the intra-group variance statistically verified. In any case it seems probable that the triploids have not a greater transpiration intensity per leaf-surface than the diploids. The correlation between the surface of the leaves and the amount of water given off during the test is very strong, the correlation coefficient for the 30 estimations, 15 for diploids and 15 for triploids, amounting to 0,847. Hence there is no great latitude for possible influences from the larger stomata of the triploids or the more numerous stomata of the diploids or for specific differences between the chromosome-number races.

If the loss of water is expressed in terms of grams per dry matter — Table 12 — there appears a greater transpiration intensity in the diploids owing to the lower dry-matter content per unit surface of these. However, this difference also is not significant.

3. *Assimilation intensity.* — The assimilation intensity was determined on severed leaves. The apparatus employed closely resembled ANDERSSON's (1944). As container a Kolle flask was used.

TABLE 10. *Sugar values in epidermis of the petioles, mol/lit.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	0,50	0,55	0,45	0,45	0,55	0,55	0,50	0,45	0,50	0,55	0,50	0,51
Block II ...	0,50	0,45	0,55	0,50	0,50	0,55	0,55	0,55	0,45	0,55	0,51	0,52
Block III...	0,45	0,55	0,55	0,45	0,45	0,40	0,60	0,50	0,40	0,60	0,49	0,50
Mean.....	0,48	0,52	0,52	0,47	0,50	0,50	0,55	0,50	0,45	0,57	0,50	0,51

TABLE 11. *Loss of water per 50 cm² leaf-surface and hour, mg.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppsmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	243	229	305	272	290	314	310	321	289	287	287	285
Block II ...	206	235	257	310	388	396	274	352	326	305	290	320
Block III...	247	289	267	311	313	302	276	281	227	306	266	298
Mean.....	232	251	276	297	330	337	287	318	281	299	281	301

TABLE 12. *Loss of water per gm dry matter, gm.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	1,30	1,07	1,73	1,40	1,29	1,15	1,69	1,66	2,19	1,36	1,64	1,33
Block II ...	1,19	1,28	1,31	1,29	1,78	1,70	1,20	1,68	1,61	1,49	1,42	1,49
Block III...	1,56	1,40	1,43	1,39	2,01	1,50	1,69	1,32	1,44	1,37	1,63	1,40
Mean.....	1,35	1,25	1,49	1,36	1,69	1,45	1,53	1,55	1,75	1,41	1,56	1,40

The duration of the experiment was 15 minutes, during which 2,10 litres of air were passed through the apparatus. This air contained about 2,5 mg. of CO_2 per litre. The temperature was 25° C, and illumination was obtained from four 200-decalumen lamps at a distance of one metre. As the determinations could not be simultaneously carried out in series for a block, leaves from two half-sibling crosses were tested the same day, determinations being made alternately on leaves from plants with a diploid father and on leaves from plants with the tetraploid father. They were made on three average leaves from each cross, and the mean values of the three determinations are given in Table 13.

TABLE 13. *Apparent CO_2-assimilation.*

Date	Mother	Father	$MgCO_2$ per 50 cm^2 leaf-area and hour	$MgCO_2$ per gm dry matter and hour
27/8	Häggeby	Hamra	2,52	8,64
		tetraploid	2,25	9,33
31/8	Sätra	Sätra	1,60	6,25
		tetraploid	1,47	6,17
1/9	Skeppmora	Sätra	1,53	5,73
		tetraploid	1,45	5,71
2/9	Ås	Ås	0,77	2,99
		tetraploid	0,88	3,27
4/1	*P. tremuloides*	Hamra	0,75	2,67
		tetraploid	0,63	2,37
	Mean	diploids	1,44	5,26
		tetraploid	1,33	5,37

No definite difference in assimilation intensity between the chromosome-number races was found. In four of the five cases the carbon-dioxide consumption per 50 cm^2 of leaf-surface was greater in the diploids, while in terms of grams per dry matter only in three cases did the diploids consume more CO_2 than the triploids. The determinations were undertaken during the period Aug. 27—Sept. 4. A remarkably rapid fall in the assimilation intensity during this short period made its appearance. The carbon-dioxide consumption the last day was not more than about one-fourth of the consumption the first day. This fact cannot possibly be interpreted as depending on any genetic difference in the experimental material, but must demonstrate a specific decline in the assimilation intensity. In fact, just about the 1st of September the height growth of the plants came to an end. The daily

growth of 45 plants was recorded during the summer, and of these 21 were still growing on Aug. 27, while 24 had already ceased to grow in height. The average growth during the 24-hour period Aug. 26—27 of the 21 still growing plants was 10,4 mm. During the corresponding period Sept. 3—4 only 7 plants were growing, with an average growth of 5,0 mm. *It is therefore clear that the assimilation intensity falls off very rapidly at the time growth in height terminates.*

IX. DRY-MATTER CONTENT OF THE LEAVES.

The measurements of dry matter made on the leaves in connexion with the investigations on the transpiration and assimilation intensity are collected in Table 14. The patch values in the table are means of two determinations. A consistently higher dry-matter content is shown by the diploids than by the triploids, and for the whole material this content is 9,8 % higher in the diploid progeny. The statistical value of the observation is fully assured; z for the difference between the chromosome races amounts to 1,3636, while P equals 0,001 for $z = 1{,}3665$.

X. QUALITY OF THE WOOD.

For the study of the character of the wood the ten largest plants of the original 12 in the fourth row of each patch were employed. The defoliated and winter-ripe plants were cut off at ground level in the middle of November, whereupon the investigations in view were immediately started. The stems were cut off at the middle, and the lower halves were used for the test. Pieces 2—3 cm. in length were cut off the tops of the barked test-samples for determination of dry-matter content and volume weight. The rest of the stems were ground for chemical analysis.

1. *Volume weight and dry-matter content.* — The volume weights are given in Table 15. Each patch value is the mean of ten determinations on as many plants. The weights were calculated on absolutely dry volume.

The reliability of the difference between the chromosome-number races is indicated by $z = 1{,}5904^{+++}$. In every instance within the triploid group there is also a significant difference, indicated by $z = 0{,}8070^{++}$, and consisting in a really lighter wood in the *P. tremuloides* offspring as compared with the heavier one of the pure aspen products. There is here an interesting agreement with previous findings.

TABLE 14. *The contents of dry matter in the leaves, per cent.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	26,47	30,60	23,74	27,52	27,84	30,08	25,71	27,23	27,28	28,83	26,21	28,85
Block II ...	28,70	27,79	26,28	29,92	30,72	28,03	28,18	29,73	26,78	29,30	28,13	28,95
Block III...	26,29	29,51	26,80	29,80	23,04	27,35	25,70	30,99	24,39	30,24	25,23	29,58
Mean.....	27,12	29,30	25,61	29,08	27,20	28,49	26,53	29,31	26,15	29,46	26,52	29,13

TABLE 15. *Specific weight of the wood.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	0,41	0,46	0,44	0,52	0,44	0,47	0,48	0,51	0,46	0,52	0,45	0,50
Block II ...	0,37	0,41	0,41	0,44	0,40	0,46	0,44	0,47	0,40	0,45	0,40	0,45
Block III...	0,41	0,44	0,50	0,46	0,39	0,43	0,47	0,51	0,43	0,47	0,44	0,46
Mean.....	0,40	0,44	0,45	0,47	0,41	0,45	0,46	0,50	0,43	0,48	0,43	0,47

TABLE 16. *Content of dry matter in the wood, per cent.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	41,9	46,4	46,0	46,8	46,1	48,2	46,3	47,3	47,7	46,1	45,6	47,0
Block II ...	42,7	43,6	45,4	44,7	42,2	45,3	45,2	47,7	44,3	46,7	44,0	45,6
Block III...	43,2	47,1	47,2	46,8	45,0	46,9	46,1	47,2	46,4	44,0	45,6	46,4
Mean.....	42,6	46,4	46,2	46,1	44,4	46,8	45,9	47,4	46,1	45,6	45,1	46,3

The volume weight for *P. tremula* is given by SIEBER (1935) as 0,45, and that for *P. tremuloides* as 0,40 by MÖRATH (1932).

When the volume weight of the wood in an absolutely dry state is known, the approximate relation between wood substance and pores (cell-lumina and intercellular spaces) can be computed. Putting a = absolute weight of the wood, b = volume of the wood, c = pore volume, and d = specific weight of the wood substance, $a = d(b—c)$ is obtained, since the pore volume is naturally devoid of absolute weight. The equation is transposed into $c = b - \frac{a}{d}$ and is divided by b, when $\frac{c}{b} = 1 - \frac{a}{b \cdot d}$ is obtained. $\frac{a}{b}$, however, is equal to the volume weight e, and as the specific weight of the wood substance is the same for practically all varieties of trees and amounts to 1,56 (HÄGGLUND, 1939), $\frac{c}{b} = 1 - \frac{e}{1{,}56}$. Hence, for the triploids, it is found that 72,48 % of the wood volume consists of pores, while the pore volume of the diploids is 69,92. Higher pore volume and lower volume weight are not, of course, a necessary consequence of the larger cells of the triploids. The absolute difference between diploids and triploids, however, is rather small. The cause of the difference is no doubt to be sought rather in the fact that the larger cells of the triploid imply larger intercellular spaces than in the fact that the cell-lumina are larger relative to the total volume of the cells.

The dry-matter content of the fresh wood is also lower in the triploids, as shown in Table 16.

For the variance due to difference in chromosome number $z = 1{,}1267^{+++}$. Hence, a larger water content is to be expected in the triploids with their larger pore volume, since most of the water is present in the pores. Still, the difference in water content, 2,7 %, is less than expected, seeing that the difference in volume weight amounts to 9,3 %. If the water stored in the cell-walls is disregarded, the following relations are obtained between the volumes of wood substance, water, and air as calculated on volume weight and dry-matter content:

	Wood substance	Water	Air	
Diploids	30,08	54,42	15,50	vol. percent
Triploids	27,52	52,26	20,22	» »

In spite of the higher water content computed on the fresh weight of the triploids these should have less water in the wood calculated on

the volume. In the diploids 77,83 % of the pore volume is filled with water, in the triploids only 72,10 %.

Cellulose, pentosan and lignin estimations were made on the samples prepared for analysis as described on page 426.

2. *Cellulose content.* — The cellulose determinations were carried out according to the technique devised by KÜRSCHNER (SCHWALBE and SIEBER, 1931) with a boiling-time of four hours. The results obtained (means of duplicate determinations) are recorded in Table 17. The triploids show a 2 % larger cellulose content, this difference being statistically unexceptionable with $z = 1{,}7442^{+++}$. It cannot, however, be decided with certainty whether a definite difference in cellulose content actually exists. The analytical procedure consists in dissolving all the substances in the wood except the cellulose. No ideal analysis would seem to exist in which all the »non-cellulose» matter is dissolved without residue but in which the cellulose is left unattacked. In these analyses, therefore, the result will depend on the length of the boiling period. To ensure reproducability the analytical conditions must therefore be constant. It is, however, conceivable that the conditions of analysis are not the same for triploids and diploids. The thicker cell-walls of the triploids may render the dissolution of the »non-cellulose» matter more difficult, and hence the triploids may show a higher cellulose value on account of an incomplete dissolution of other substances. The higher cellulose content registered in the analyses on the triploids may be a direct result of the thicker cell-walls and the consequent greater resistance offered to the action of chemicals. On the other hand, of course, there may be a higher specific cellulose content.

3. *Pentosan content.* — The furfurol content corresponding to the pentosan content was estimated by TOLLEN's method (SCHWALBE and SIEBER, 1931 and Tekn. medd. Sv. pappers- o. cell.-ing.-för. CCA 4), and the results are recorded in Table 18. Quite the greater portion of the pentosan is probably present in the form of xylane, and in that case the pentosan content would be given by the formula furfurol content $\times$ 1,55. The triploids should thus possess a pentosan content of 19,84 % and the diploids 20,12 %. That small difference is, however, without statistical significance.

4. *Lignin content.* — The lignin content of the wood has been determined by breaking it down with 72 % sulphure acid according to the method described in Tekn. medd. Sv. pappers- o. cell.-ing.-för. CCA 5. The results of the analyses are given in Table 19. All differences are small and entirely insignificant.

TABLE 17. *Contents of cellulose, per cent of dry matter.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	51,29	51,21	49,11	48,96	50,87	50,61	47,22	47,05	49,28	49,10	49,55	49,39
Block II ...	54,10	50,85	53,35	49,25	52,04	49,31	53,37	52,11	49,44	48,57	52,46	50,02
Block III...	52,19	51,10	50,50	49,40	50,95	52,22	51,52	50,91	50,36	49,22	51,10	50,57
Mean.....	52,53	51,05	50,99	49,20	51,29	50,71	50,70	50,02	49,69	48,96	51,04	49,99

TABLE 18. *Furfurol, corresponding to the amount of pentosan, per cent of dry matter.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	13,00	12,39	12,82	13,39	12,94	12,31	12,60	13,26	12,91	13,60	12,85	12,99
Block II ...	12,38	12,99	12,73	13,55	12,67	12,28	13,02	12,94	13,34	13,42	12,83	13,04
Block III...	12,87	12,77	12,98	13,11	12,24	12,63	12,84	13,23	12,63	12,82	12,71	12,91
Mean.....	12,75	12,72	12,84	13,35	12,62	12,41	12,82	13,14	12,96	13,28	12,80	12,98

TABLE 19. *Contents of lignin, per cent of dry matter.*

Mother	*P. tremuloides*		Sätra		Häggeby		Skeppmora		Ås		Mean	
Father	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Hamra	tetrapl.	Sätra	tetrapl.	Ås	tetrapl.	diploids
Block I.....	19,67	20,86	20,82	19,99	20,21	19,95	20,15	20,14	20,35	20,90	20,24	20,37
Block II ...	18,79	20,07	20,13	20,79	20,27	20,22	18,79	20,16	20,54	19,65	19,70	20,18
Block III...	19,90	20,11	19,55	21,01	20,48	19,99	19,45	19,70	20,23	19,66	19,92	20,10
Mean.....	19,45	20,35	20,17	20,50	20,32	20,05	19,43	20,00	20,37	20,07	19,95	20,21

XI. THE VALUE OF THE RESULTS FOR THE ESTIMATION OF THE FUTURE DEVELOPMENT.

The results submitted above have reference to one-year plants. An extremely interesting question is whether those relations between diploids and triploids which have appeared in the yearlings will also be retained in the adult trees at 40—50 years of age. With the guidance of the experiences at hand from adult spontaneous triploid aspens certain reflections may be made. The stomata of the yearlings were 26 % longer in the triploids. The stomata of adult triploids are longer by 10—25 % than those of diploids from the same localities. It is therefore probable that the numerical relation between the mean sizes of the stomata will remain moderately unaltered. The increase in the size of the wood-cells in all trees follows a curve that ascends fairly evenly up to maximum dimensions, which is attained at 30—40 years of age (TRENDELENBURG, 1939; T. NILSSON, 1943). It is natural to assume that the same will also take place here. The average length of the wood-cells in the yearlings was 0,56 mm. in the triploids and 0,48 mm. in the diploids. Preliminary investigations on adult trees have given the values 1,28 mm. for triploids and 1,18 mm. for diploids (JOHNSSON and NILSSON, unpubl.). That the triploids will always possess larger cells than the diploids is incontestable. The investigation has shown that the triploid yearlings have considerably larger leaves than the diploids. The spontaneous triploid trees have just their exceptionally large leaves to thank for their discovery (NILSSON-EHLE, 1936; JOHNSSON, 1940). There are therefore good grounds for assuming that the difference in leaf-size will be permanent.

As regards growth of the stem, no effect from the degree of polyploidy could be detected in the yearling plants. What the actual conditions are in the spontaneous triploids is often difficult to judge, as not uncommonly diploid trees of comparable value are absent in the vicinity of the triploids. Statements are met with to the effect that the triploids grow more rapidly (NILSSON-EHLE, 1936). Some possibility of making a more detailed estimate is afforded by PETRINI (1944). PETRINI has measured the growth of aspens in three different test-areas at Sätra Bruk in Västergötland, viz. areas Nos. 376, 377 and 378. Areas 377 and 378 are covered with the same triploid clones (NILSSON-EHLE, JOHNSSON, unpubl.), while No. 376 is covered with diploid aspens. PETRINI finds an 11 % greater height and a 10 % greater diameter for the triploid trees. During the past 56—57 years the

production per unit area has been 36 % larger for the triploid, the actual growth of which keeps a lead of 18 %. Consequently, there seems to be reason to assume that the retardation in the rate of division which apparently takes place in the cambium of the triploid yearlings as compared with the diploid may gradually diminish or cease.

The triploid yearlings showed a wood volume weight 9 % lower than that of the diploids, the absolute values being 0,43 and 0,74 respectively. A limited adult material that was examined gave a volume weight of 0,49 for triploids and 0,53 for diploids, a difference of 8 %. It therefore seems probable that in the future the triploids will continue to possess a lighter wood than the diploids. In the case of the other properties investigated no definite evidence was obtained as to the influence of the degree of polyploidy. Whether these results indicate permanent relations can only be decided by future investigations in the course of years.

XII. DISCUSSION.

Observations of gigantism in autopolyploids have been published in numerous cases ever since GATES (1909) established tetraploidy in *Oenothera Lamarckiana gigas*. However, especially since colchicine treatment was introduced as a simple and effective method of producing polyploids, it has become clear that this *gigantism is not an obligatory and total character in autopolyploids*. Still, in the majority of autopolyploids there is a partial gigantism. This manifests itself in the first place in an increased cell-size. In practically all cases examined there is a more or less pronounced increase in the size of the cells in autotri- and tetra-ploids. In the present aspen material the diameter of the pollen-grains is 9 % larger in triploids than in diploids, and the tetraploid examined possesses a further 12 % larger grain diameter than the triploids. The stomatal cells were 26 % longer in the triploids than in the diploids. The wood-cells in the xylem of the triploids surpassed those of the diploids in length and breadth by 17 % and 20 % respectively, and in volume by 74 %. This increase in cell-size is to be regarded as the primary effect of the polyploidy, the cell-plasma ratio according to WEISMANN. Unless the polyploidy brings about some other changes, the result must be a general gigantism. That this is not always the case has been contended of late years by several investigators, e. g. PIRSCHLE (1942), LEVAN (1945), and is brought out further by the present material. In the triploid yearlings of *Populus tremula*

it is, besides the cell-size, only the leaves which display distinct gigantism. The triploids have a leaf-surface that is 56 % larger than that of the diploids. Their leaves are 14 % thicker and their leaf-volume 77 % larger, which is in close agreement with the increase in cell-volume of 74 % found in the wood-cells of the stem. The increase in leaf-size may thus be considered to be directly proportional to the increase in cell-size. Increased size of leaf is also the commonest secondary effect of polyploidy. Thus, in comparisons between 12 different autotetraploids and their original forms, PIRSCHLE (1942) found leaf gigantism in all cases.

There was no increase in growth of the stem corresponding to the gigantism of the leaves. On the contrary, the triploids showed somewhat inferior — although not significantly inferior — growth in length and diameter. In PIRSCHLE's above-mentioned material the polyploidy was likewise unattended by any general increase in plant height. Only in two cases did the tetraploids show greater plant height. In two cases there was no difference in this respect, and in the remaning eight cases the height was lower in the polyploids.

To explain why gigantism is sometimes present, sometimes not, it has for long been assumed that polyploidy often brings about a retarded rate of cell division which counteracts the increase in cell-size. This retardation may be different in different organs. HEILBORN (1933), for instance, pointed out that the deviations from the normal number of cells are smaller in organs with limited growth (e. g. leaves) than in organs with unlimited growth (stems). This is considered to be explained by the fact that organs with limited growth are differentiated early during the period of vegetation. The maximum number of cell divisions under the prevailing conditions is also completed in the polyploids in spite of any lower rate of division that may occur, while in organs that grow during the whole period of vegetation the number of cell divisions is lower in the polyploids. However, the polyploids often seem to possess a longer period of vegetation (see ANDERSSON, 1943, and the literature cited by him). In such cases the difference in the number of cells ought to be evened out and organs with unlimited growth should also show gigantism. There was no difference in time of cessation of growth in the autumn between the diploid and triploid aspen families. The growth in length stopped at the same time and the leaves were shed at the same time. A measurement of the plant height undertaken on Aug. 1st gave results which were practically absolutely correlated to the height in the autumn. Although it cannot

be considered as demonstrated that polyploidy causes a general and specific reduction in the rate of cell-divisions, there should be good grounds for assuming that this is one of the commonest among the secondary effects of polyploidy.

Naturally enough, differences in the speed of the cell-divisions may directly depend on changes in the regulatory mechanism of the plant. The speed of cell-divisions may, however, be dependent on the quantity of assimilates available at each point and zone of vegetation. It would therefore be of the greatest interest to know whether polyploidy brings, as a secondary effect, any alterations in the assimilatory capacity of the leaves. Direct determinations of the intensity of assimilation in diploids and polyploids are only available for a few plants. Diploid and tetraploid *Hordeum vulgare* has been studied by ANDERSSON (1943), EKDAHL (1943), and STÅLFELT (1943). ANDERSSON found a lower assimilation per 50 cm^2 of leaf-surface in the tetraploid, EKDAHL and STÅLFELT a somewhat higher. In *Trifolium repens* STÅLFELT also found a slightly larger consumption of CO_2 per unit surface. Lower assimilation values per unit of surface were registered in *Solanum nodiflorum* (LARSEN, 1941), *Sinapis alba* (LARSEN, 1943), and in *Phleum nodosum* (STÅLFELT, 1943). In the present aspen material there was a somewhat lower assimilation per unit surface in the triploids. Per gram fresh weight the assimilation was lower in the polyploids in all the cases examined, and per unit dry weight lower except in *Phleum nodosum* and *Populus tremula.*

The chief cause of the consistently lower assimilation per unit weight of fresh substance is the invariably larger water content possessed by the leaves of the polyploids. This is the case with the aspens in question here and has been previously shown in other objects of investigation by, among others, MÜNTZING (1940), SCHLÖSSER (1937), LEVAN (1943), ANDERSSON (1943), STÅLFELT (1943), and EKDAHL (1943). A larger water content in the polyploids would thus seem to be an almost invariable secondary effect of polyploidy. In *P. tremula* the water content is larger in the polyploid even in the wood, although the difference there is less than in the leaves. That the assimilation intensity calculated on dry substance is mostly still more unfavourable for the polyploids than when computed per unit surface depends on the fact that as a rule the polyploids possess a larger quantity of dry substance per unit of surface on account of their thicker leaves. Data presented by LEVAN (1943) show that this was so in seven cases. *Populus tremula*, however, shows on both fresh and dry substance a

lower assimilation per unit surface in the triploid than in the diploid. The fresh weight per cm^2 in the present material is 16,4 mg. for the triploids and 16,8 mg. for the diploids. For the dry matter the corresponding values are 4,34 mg. and 4,88 mg. per cm^2. *Populus tremula* was also the only exception in LEVAN's material. This explains why the assimilation intensity calculated on dry substance works out somewhat higher for the triploid aspen. The as yet fragmentary data available concerning the relative assimilations of diploids and polyploids do not allow of any generalizations. For the development of the plant the maximum assimilation intensity is of limited value. The effective daily assimilation period is undoubtedly more important, and on this point no investigations are available.

LEVAN's above-cited work furnishes strong evidence of there being a lower pigment content in the polyploids per unit surface, per unit fresh substance, and per unit dry matter, and the author puts forward the hypothesis that this is due to the larger amount of substance per unit surface consequent upon the thicker leaves, considering that this results in a smaller amount of pigment because the pigment concentration diminishes towards the centre of the leaves. Within *Populus tremula*, both in diploids and triploids, there is a distinct negative correlation between leaf-thickness and stem-volume. The correlation coefficient is $-0{,}5621$ with $t = 2{,}451^{+}$ for the diploids and $-0{,}7199$ with $t = 3{,}754^{++}$ for the triploids, calculated on the 15 pairs of patch values within each category. The regression constant is $-1{,}24$ with $t = 1{,}865$ for the diploids and $-0{,}87$ with $t = 2{,}762^{++}$ for the triploids. As a regression constant common to both $-1{,}03$ is obtained, to which corresponds $t = 3{,}054^{++}$. The regression between the chromosome-number groups is only $-0{,}25$, however. The correlation between increasing leaf-thickness and decreasing stem-volume within the chromosome-number races would thus seem, in cases of increased leaf-thickness due to polyploidy, to be counteracted by some factor or other which tends to increased volume. This other factor may undoubtedly be sought in the larger cells of the polyploid. As pointed out above, however, the increased leaf-thickness brought about by polyploidy is not associated in *P. tremula* with an increase in the amount of substance per unit of leaf-surface but with a slight diminution instead. None the less, increase in leaf-thickness due to polyploidy or to recombination variation seems to be unbeneficial for the growth of the stem. As the increasing leaf-thickness conditioned by polyploidy must be regarded as a direct consequence of the increase in the size of the cells, it is likewise im-

probable that the increase in leaf-thickness is a hypertrophic phenomenon consequent upon feeble growth and low consumption of assimilates in the stem, with an excess of assimilates in the leaves as a result.

The osmotic pressure has been found to be lower in a large number of polyploids than in the primary forms (BECHER, 1932; SCHLÖSSER, 1936; GYÖRFFY, 1941 a). However, the results are not uniform (ANDERSSON, 1943). No differences have been detected in *Populus tremula*. As a distinct secondary effect of the polyploidy in *Populus tremula* there is the diminution in the volume-weight of the wood. This diminution, which amounts to 9 %, corresponds to a 4 % larger pore volume. This is probably due more to larger intercellular spaces in the triploid than to altered relations between cell-lumina and walls. In the diploids 77,83 % of the pore volume is filled with water, but in the triploids only 72,10 %. This is undoubtedly a result of a lower capillary action of the larger pores in the triploids.

Comparative investigations of the chemical composition of diploids and polyploids have been performed by several research-workers, e. g. GREIS (1940), MÜNTZING (1940), GYÖRFFY (1941), EKDAHL (1943), JULÉN (1944). At times considerable differences have been observed, in the other respects there have been no differences. No uniform tendencies can be elicited from the facts. In *Populus tremula* no differences have been detected in the lignin and pentosan contents. On the other hand, the triploids gave a higher value for cellulosa. This difference, however, is probably only apparent and depends on the greater resistance of the thicker cell-walls in the triploid to the action of chemicals.

In yearlings of the triploid aspen, therefore, the following changes evoked by the polyploidy have been detected: A primary effect in the shape of increased cell-size and secondary effects in the form of gigantism of the leaves, higher water content of leaf and wood, lower volume weight of the wood (also of the leaves), as well as a higher cellulose content of the wood, although this effect is probably only apparent. Leaf gigantism and higher water content have been so universally found in autopolyploids that these properties can be denoted as the commonest secondary effects of the polyploidy. An interesting though — from a practical point of view — negative fact is that no gigantism appeared in the stems of the yearlings. It must, however, be regarded as a likely possibility that the stem will also exhibit gigantism when the trees become older, since it is a very common

observation that the polyploids develop more slowly than the diploids. A phase displacement in development may therefore also be expected on good grounds in *Populus tremula*, and should then manifest itself in the polyploids growing at an older age, after the diploids have already ceased growth, and finally developing into larger trees. As a result, the partial gigantism marking the yearlings would pass into a more total gigantism. A development of this kind, »entwicklungsphysiologischer Umschlagspunkt» (SCHLÖSSER, 1940), may also take place through an accelerated rate of division in the cambium of the polyploids in older years.

SUMMARY.

(1) An investigation has been made on yearling plants of *Populus tremula* belonging to five diploid and five triploid cross-families with, in pairs, the same diploid mothers and diploid or tetraploid fathers.

(2) The following properties have been studied: leaf-size, leaf-thickness, stomatal length, number of stomata per leaf-surface, length of wood-cells, diameter of wood-cells, growth in length, diameter and volume of stem, osmotic pressure, transpiration and assimilation intensity, dry-matter content of the leaves, volume weight and dry-matter content of the wood, the cellulose, pentosan and lignin contents of the wood.

(3) A primary effect of the polyploidy is increased cell-size, which was found in pollen-grains, stomatal cells and the wood-cells in the xylem.

(4) As secondary effects of the polyploidy leaf gigantism, increased water content, and reduced volume weight were observed.

(5) The polyploidy has not caused any gigantism in the stems of the young plants, although it is considered probable that such may appear in the older trees.

(6) No specific influences from the polyploidy have been detected on the physiological and chemical properties studied.

(7) The results of the investigation are discussed, special stress being laid on the fact that a negative correlation exists between leaf-thickness and growth of stem-volume.

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LITERATURE CITED.

1. ANDERSSON, G. 1943. Vergleichende Untersuchungen der Assimilationsintensität diploider und tetraploider Gerste. — Svensk Bot. Tidskr. 37.
2. — 1944. Gas change and frost hardening studies in winter cereals. — Lund.
3. BECHER, G. 1932. Experimentelle Analyse der Genom- und Plasmonwirkung bei Moosen. III. Osmotischer Wert heteroploider Pflanzen. — Zschr. f. ind. Abst.- u. Vererb.-Lehre 60.
4. BERGSTRÖM, I. 1940. On the progeny of diploid × triploid *Populus tremula*. With special reference to the occurrence of tetraploidy. — Hereditas XXVI.
5. EKDAHL, I. 1943. Comparative studies in the physiology of diploid and tetraploid barley. — Arkiv för Bot. 31 A.
6. FISHER, R. A. 1938. Statistical methods for research workers. — Edinburgh.
7. GATES, R. R. 1909. The stature and chromosomes of *Oenothera gigas*, DE VRIES. — Arch. f. Zellforsch. 3.
8. GREIS, H. 1940. Vergleichende physiologische Untersuchungen an diploiden und tetraploiden Gersten. — Der Züchter 12.
9. GYÖRFFY, B. 1941 a. Untersuchungen über den osmotischen Wert polyploider Pflanzen. — Planta 32.
10. — 1941 b. The physiological and chemical condition in polyploid plants. — Magyar Biol. Kut. Munk. XIII.
11. HEILBORN, O. 1933. Über Eutelie oder Zellkonstanz in pflanzlichen Geweben. — Svensk Bot. Tidskr. 27.
12. HÄGGLUND, E. 1939. Holzchemie. — Leipzig.
13. JOHNSSON, H. 1940. Cytological studies of diploid and triploid *Populus tremula* and of crosses between them. — Hereditas XXVI.
14. — 1942. Generativ och vegetativ förökning av *Populus tremula*. — Svensk Bot. Tidskr. 36.
15. JULÉN, G. 1944. Investigations on diploid, triploid and tetraploid lucerne. — Hereditas XXX.
16. LARSEN, P. 1941. Vergleich der direkt bestimmten und der aus Messungen der Assimilation und Atmung errechneten Stoffproduktion einjähriger Pflanzenbestände. — Planta 32.
17. — 1943. The aspects of polyploidy in the genus *Solanum*. — Det Kgl. Danske Vidensk.-Selsk. Biol. Medd. 18.
18. LEVAN, A. 1943. The pigment content of polyploid plants. — Hereditas XXIX
19. — 1945. Aktuelle Probleme der Polyploidiezüchtung. — (In the press.)

20. MÜNTZING, A. 1936. The chromosomes of a giant *Populus tremula*. — Hereditas XXI.
21. — 1940. Further studies on apomixis and sexuality in *Poa*. — Hereditas XXVI.
22. MÖRATH, E. 1932. Studien über die hygroskopischen Eigenschaften und die Härte der Hölzer. — Mitt. d. Holzforschungsstelle a. d. T. H. Darmstadt 1.
23. NILSSON, T. 1943. Skogsträdsförädling enligt kemiskt kvantitativa riktlinjer. — Svensk Papperstidning.
24. NILSSON-EHLE, H. 1936. Über eine in der Natur gefundene Gigasform von *Populus tremula*. — Hereditas XXI.
25. — 1938. Framställning av skogsträd med ökat kromosomtal och ökad virkesproduktion. — Svensk Papperstidning.
26. PETRINI, S. 1944. Tre försöksytor i aspskog. — Medd. fr. Statens Skogsförsöksanstalt 34.
27. PIRSCHLE, K. 1942 a. Quantitative Untersuchungen über Wachstum und »Ertrag» autopolyploider Pflanzen. — Zschr. f. ind. Abst.- u. Vererb.-Lehre 80.
28. — 1942 b. Weitere Untersuchungen über Wachstum und »Ertrag» von Autopolyploiden (2n, 3n, 4n) und ihren Bastarden. — Zschr. f. ind. Abst.- u. Vererb.-Lehre 80.
29. SCHLÖSSER, L. A. 1936. Frosthärte und Polyploidie. — Der Züchter 8.
30. — 1937. Grenzen und Möglichkeiten der Ausnutzung von Polyploidie in der Pflanzenzucht. — Forschungsdienst 3.
31. — 1940. Physiologische Untersuchungen an polyploiden Pflanzenreihen. — Forschungsdienst 10.
32. SCHWALBE, C. G. und SIEBER, R. 1931. Die chemische Betriebskontrolle in der Zellstoff- und Papier-Industrie. — Berlin.
33. SIEBER, R. 1935. Über das spezifische Gewicht von Zellstoffholz. — Papierfabrikant 33.
34. STÅLFELT, M. G. 1943. Kohlensäureassimilation und Atmung der grosswüchsigen Polyploiden. — Arkiv för Bot. 30 A.
35. SYLVÉN, N. 1940. Lång- och kortdagstyper av de svenska skogsträden. — Svensk Papperstidning.
36. TRENDELENBURG, R. 1939. Das Holz als Rohstoff. — Berlin.

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INHERITANCE OF PNEUMATISATION OF THE MASTOID BONE

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INTRODUCTION.

IT has long been known that the pneumatisation of the mastoid bone may display very varying degrees of development, as also that, in chronic otitis, the cellular system as a rule is only slightly developed. Some authors have also been of the opinion that acute otitis is more probable when the cellular system is large. The size of the cell systems has been estimated from anatomical preparations and roentgenograms, and approximately classified as small, medium, large, etc. Naturally, it has not in so doing been possible to set limits according to the same standards; the figures obtained by different authors from estimates of this kind have therefore shown great differences. It was not until DIAMANT's investigation (1940) that exact and numerical data were obtained.

DIAMANT's method was to measure with a planimeter the surface shown by the cellular system on a sagittal roentgenogram. A special investigation revealed a very strong correlation between the extent of the cellular system in the sagittal and the frontal plane. A closer examination of the sources of error attaching to the method showed great precision to have been obtained. Thanks to DIAMANT's investigation it has been possible to obtain exact normal measurements of the size of the cellular system and of the variability round the average values for adult men and women. It has furthermore been possible to show that, in chronic otitis, the average size of the cellular system amounts to a quarter of the mean for adults. The cellular system of individuals with chronic otitis almost never reaches the mean for normal ears. Finally, it has been shown that, in acute otitis, the cellular system is, on an average, three-quarters as big as the average system in adults. In other words, the risk of acute otitis is somewhat, and the risk of chronic otitis materially, greater for persons with small cellular systems than for those with large. We may even say that persons with particularly large cellular systems may, although rarely, contract acute otitis, but that chronic otitis practically never develops from it.

Under such circumstances, it is obviously interesting to try to find out how far the size of the cellular system is conditioned by hereditary factors. There are only a few investigations touching on this problem. They have, throughout, been made on an approximate assessment of the size of the cellular system. The information obtained is therefore very vague.

SURVEY OF THE LITERATURE.

LEICHER (1928) has investigated 39 pairs of monozygotic twins, and considered himself able to show pneumatisation differences in 36 %. At the assessment, only inhibited and non-inhibited cellular systems were distinguished. Figures of this kind indicate that hereditary factors are of importance. However, the data cannot but be very subjective. The procedure corresponds in a sense to an investigation into the inheritance of stature in which one only distinguishes between small persons with arrested stature, and others, then being content to judge the size by eye.

In SCHWARZ's investigation (1929) of 61 pairs of monozygotic twins and 35 pairs of dizygotic twins, roentgenograms showed the cell systems to agree in size in 66,1 % of the monozygotes, and 37,1 % of the dizygotes; he considers this to indicate that factors of heredity play a dominant part. When assessing agreement, however, regard has been paid not only to the size of the cell system but also to the thickness of the walls and the more or less regular arrangement, etc., of the system. Thus, subjective factors have to a certain extent influenced the decision as to 'similarity' or 'dissimilarity'. In other words, it is impossible to say definitely what figures other investigators would have obtained from a study of the same material.

OKASAKI (1935) has investigated 18 pairs of monozygotic twins and 11 pairs of dizygotes for pneumatisation in the mastoid bone. He, too, has taken into account the size of the cell walls and the arrangement, etc., of the cells. He finds agreement in 75 % of the monozygotes and in 45 % of the dizygotes. The differences between his figures and those obtained by SCHWARZ may well exemplify the subjectivity behind the judgment as to similarity and dissimilarity. It is surprising that he reports the four ears in a pair of monozygotic twins to be so alike as to invite confusion; this does not agree with our experiences from our own material.

SCHWARZ (1937) has furthermore investigated a family for the size and build of the cellular system, and considers himself able to deduce

from the roentgenograms of mother and children (father not investigated) a dominant inheritance for small systems. In point of fact, of course, the material allows of no conclusions. SCHWARZ has gone further and investigated families with probands, who had had acute or chronic scarlatina otitis as children. His intention was to discover whether a sustained otitis inhibits the development of the cell system, WITTMACK having advanced the hypothesis that small cell systems are due to an early otitis. In the 11 cases who had had otitis before the age of 5 years, 7 had little or no pneumatisation. In the families of 3 of these cases, however, SCHWARZ found that siblings also showed small cellular systems, and considered the material to be still indicating dominant inheritance. The material SCHWARZ published is unfortunately not large enough to allow of conclusions.

DILLON and GOUREVITCH (1936) have investigated 11 pairs of monozygotic and 11 pairs of dizygotic twins. They submit only approximate data as to similarity and dissimilarity. The sole distinction they make is between a well-pneumatised and a sclerosed mastoid process. They draw the conclusion that differences in pneumatisation are mainly due to environment. The investigation shows that very divergent conclusions may be reached when subjective factors are allowed to decide the issue (cf. SCHWARZ above). It is only possible to solve the problems with an exact method of investigation.

On the whole, however, the investigations which have been made indicate that hereditary factors do have something to do with determining the size of the cellular system in different individuals. But it is necessary that the problem be submitted to a more detailed analysis.

There are no investigations showing that a liability to chronic otitis is more or less inherited. STEIN (1917) has tried to show that a tendency to ear complaints of different kinds is hereditary to a certain extent. The fundamental line he follows is that this depends on the »inheriting of inferior organs», an opinion which, incidentally, has been advanced earlier by other authors (ADLER). He submitted a number of cases of ear complaints in which the patients showed a large number of degenerative stigmata. He considered these so-called signs of degeneration to bear out his view. The cases included both acute and chronic otitis. When he speaks of inferior organs, he does not refer merely to the actual auditory system, but to all organs. In other words, the inferiority of the auditory organs has its root in a general degenerative constitution. The selected cases published in his work have hardly any

value as proof, and his theoretical opinions, which follow on from older ideas of heredity, are no longer of interest.

In a later work by BAUER and STEIN (1925) an investigation is made of the frequency in the family of cases of labyrinthine deafness and otosclerosis. They further investigate individuals with chronic inflammation of the middle ear; these persons are in this paper regarded as control material, however. Figures for relations (siblings, parents) of this latter group indicate that heredity plays some part, but the two authors have not analysed the group in any great detail. The investigation aims at establishing the details of the hereditary mechanism behind otosclerosis and labyrinthine deafness. The data as to chronic inflammations of the ears are not sufficiently minute to allow of very definite conclusions.

Investigations of monozygotic twins have greater value. WEITZ (1925) described 3 such pairs in which both twins suffered from inflammation of the ear. Two similar observations have been published by PAULSEN (1925). These 5 cases are nevertheless not enough to warrant conclusions being drawn; they may be selected chance coincidences. Following on from the WITTMACK theory as to the nature of the mucosa in the cell system, ALBRECHT (1931) and SCHWARZ (1935) have tried to show that chronic otitis is conditioned by special genes. The latter author even goes so far as to assert that the disease is dominant in certain families. Both these authors base their statements on observations from families in which inflammations of this kind showed a high frequency. Since diseases of this kind are not uncommon, the few family-trees which have been published are not much use as proof. The possibility of chance coincidences cannot be ruled out.

The view these authors advance is, in principle, not altogether unlike the viewpoints applied here. According to WITTMACK, hyperplastic and atrophied mucosa in the cell system both inhibit its development and prompt chronic inflammation of the ear. SCHWARZ and ALBRECHT assume that such mucosal anomalies are hereditary, while WITTMACK is of the opinion that inflammatory injuries at an early stage are the real cause. Now, we cannot investigate the mucosa of living persons still in health to show that those presenting certain anomalies later on contract chronic inflammation of the ear to a greater extent than those with normal mucosa. Observations can only be made when operating upon persons displaying symptoms of the disease, and it is then not possible to decide whether an abnormality in the mucosa is caused by the disease, or whether it was present earlier. The fact that normal

individuals with no inflammation of the ear may reveal a thin, so-called atrophic or hyperplastic mucosa does not permit of any conclusions as regards their risk of disease. It is impossible to obtain material for comparison purposes, or to reach any figures at all warranting conclusions. The WITTMACK theory is simply a construction based on chance observations and impressions.

We are, on the other hand, on firm ground if we content ourselves with investigating the size of the cell system by roentgen, in the way done here. DIAMANT's investigation shows definitely that the size of the cell system has a bearing on the risk of chronic otitis. It is possible that qualitative differences in the mucosa of the cell system also run parallel with differences in its size. It seems natural to assume that a growth in the mucosa is the primary cause of small cell systems, and that a mucosa with feeble growth may, in other respects also, show differences (most likely atrophic in type; hardly hyperplastic). Other factors, such as the development of the vascular system, might also be thought to play a part. This side of the question is of less importance from a practical point of view, however. What is important is the connection between chronic inflammation of the ear and a small cell system, since both these can be observed, whereas qualitative differences in the mucosa or the vascular system cannot.

THE PROBLEM.

Under these circumstances it is natural, when discussing the importance of hereditary factors for otitis, to connect up with the size of the cell system. It can then be assumed that acute inflammation of the ear is not conditioned by heredity. Granted that small cell systems render the ears somewhat more liable to acute inflammation than large ones, the extent to which they do so is nevertheless so small that it can hardly be thought to provide a hereditary background to any appreciable degree. The situation is different in regard to chronic otitis. Since this complaint only appears in persons with small cell systems, it is obvious that, if hereditary factors decide the size of the cell system, they must also decide the occurrence of chronic otitis. Needless to say, this does not rule out environmental influence. Not everyone with a small cell system contracts acute otitis, and if he does, it does not always become chronic. No doubt the situation is that certain genes which determine the size of the cell system, occasion thereby a disposition to otitis. Environmental factors which decide whether an infection is to arise subsequently determine the issue. In these circum-

stances chronic otitis will, to use DAHLBERG's terminology, be a *constellational* character, due to a meeting of hereditary factors, only present in some of the population, with environmental factors, also only present in some of the population. In marginal perforation in particular, the possibility of a cholesteatoma must be taken into account. Cases with cholesteatoma may, of course, show a different state of affairs; environmental factors may then have played a relatively small part. The problems here are linked up with the question of the etiology of the cholesteatoma. An assessment of the parts played by heredity and environment respectively calls for special investigations in each separate type of cases. Our intention in the work presented here is to discover how far hereditary factors affect the size of the cell system. The account given above is only intended to explain our approach.

The material used in the present investigations consists partly of pairs of monozygotic and dizygotic twins, partly of families comprising one child and the two parents. The individuals have been investigated according to the methods worked out by DIAMANT (1940) — that is to say, photographed with roentgen, after which the size of the cell system is determined on the roentgenogram with a planimeter.

CORRELATION BETWEEN PARENTS AND CHILDREN AS REGARDS THE SIZE OF THE CELL SYSTEM.

When collecting the family material, 301 children were used to start the investigation. The age distribution is seen from Table 1.

TABLE 1. *The family material. Age (years completed) of the children at the examination.*

Age	Number of children
12	20
13	120
14	104
15	43
16	11
17	1
Unknown	2
Total	301

The boys were all over 13 years of age, and the girls all over 12. It can safely be assumed that the cell system has reached its final size at these age limits. The median for the age was 14,3 years for boys,

and 14,0 years for girls. Both the father and the mother of these initial subjects were investigated.

The size of the cell system in both children and parents is shown in Table 2.

TABLE 2. *The family material. Size of the cell system (in cm²) in parents and children.* [$M \pm \varepsilon(M)$ = *mean* ± *standard error of the mean.* σ = *standard deviation.*]

	Number	M ± ε (M)	σ
Mean of right and left			
Boys	137	14,03 ± 0,49	5,78
Girls	164	14,03 ± 0,36	4,66
Father	301	11,70 ± 0,31	5,36
Mother	301	11,99 ± 0,30	5,14

The figures agree with DIAMANT's normal figures of 12,27 cm², with a standard deviation of 5,4 cm² in the age group 10—30 years. It is possible that the cell system gets somewhat smaller with advancing age, and that this explains the tendency to differ which is found between children and parents in our material. It may also be that the difference we found is due to the difference in general size between generations, now obtaining in Sweden as a result of increasing stature.

The crucial question is whether a connection can be established between the size of the cell systems in children and parents. To begin with, Table 3 gives figures showing the size of the cell system in either parent for different sizes of the same system in the child. Using median and quartiles, boys and girls alike have been divided up into 4 equal groups.

The smaller the cell systems of the child, the smaller those of the parents, and *vice versa.* The difference between the groups of parents is smaller than that between the corresponding groups of children. There is a regression towards the mean when passing from child to parent — not, in itself, a remarkable circumstance. Similar phenomena have been observed for other qualitative characters, e. g. stature.

To clarify the situation more satisfactorily, we now give, in Table 4, figures of the correlation between parents and children.

We find throughout a statistically significant correlation coefficient, amounting to about + 0,40. The correlation between girls and their fathers happens to be small. In view of the size of the standard error, however, the low figure may be attributed to chance. There is thus a plain correlation between parents and children, and the most likely explanation of this seems to be that hereditary factors are at work.

TABLE 3. *The average size of the cell system in mothers and fathers respectively of children with cell systems of different sizes. The children are divided into 4 groups: (1) Cell system < first quartile. (2) Cell system between first quartile and median. (3) Cell system between median and third quartile. (4) Cell system > third quartile.* [$M \pm \varepsilon(M)$ = *mean* ± ± *standard error of the mean.*]

Groups with regard to the cell system in the children		Cell system of the mother		Cell system of the father	
		Number	M ± ε (M)	Number	M ± ε (M)
Boys:					
1 quartile	< 10	34	9,24 ± 0,83	34	9,33 ± 0,86
2 »	10 —13,4	35	10,91 ± 0,88	35	10,83 ± 0,73
3 »	13,4—17,9	34	12,99 ± 0,82	34	11,46 ± 0,82
4 »	> 17,9	34	15,48 ± 0,92	34	14,63 ± 1,01
Girls:					
1 quartile	< 11,1	41	10,38 ± 0,83	41	10,82 ± 0,93
2 »	11,1—13,9	41	11,03 ± 0,61	41	12,02 ± 0,72
3 »	13,9—17,1	41	11,72 ± 0,74	41	12,03 ± 0,85
4 »	> 17,1	41	14,31 ± 0,70	41	12,42 ± 0,88
Boys and Girls:					
1 quartile	.....	75	9,87 ± 0,58	75	10,14 ± 0,64
2 »	.....	76	10,97 ± 0,52	76	11,47 ± 0,51
3 »	.....	75	12,29 ± 0,54	75	11,77 ± 0,59
4 »	.....	75	14,84 ± 0,56	75	13,42 ± 0,67

TABLE 4. *Correlation between the size of the cell system in children and parents.* [$r \pm \varepsilon(r)$ = *coefficient of correlation* ± *standard error.*]

	Number	r ± ε (r)
Boys — father	137	+ 0,39 ± 0,073
Girls — father	164	+ 0,13 ± 0,077
Boys — mother	137	+ 0,42 ± 0,071
Girls — mother	164	+ 0,37 ± 0,067
Boys + girls — father	301	+ 0,26 ± 0,054
Boys + girls — mother	301	+ 0,40 ± 0,049
Father — mother	301	+ 0,074 ± 0,057

The correlation between the parents has also been calculated, for purposes of checking. There is none — nor, indeed, was any to be expected.

We must now consider the possibility of both parents having cell systems of different sizes. The degree of such differences must have an effect on the correlation coefficients obtained. Logically, we should expect the correlation to be far greater if both parents had the same

sized cell system than if large differences were present. The material is not large enough to enable us to make a detailed analysis; we confine ourselves to dividing the parents into two groups, according to the differences between them. These groups contain those with differences respectively larger and smaller than the median. As expected, we now find a higher correlation for the group with relatively similar parents, amounting to $+ 0{,}51 \pm 0{,}061$; on the other hand, no significant correlation is obtained when the parents are unlike one another. In this latter case the children will agree now with the one parent, now with the other, and now show values in between, and it is natural that no significant correlation is obtained between the means of the parents and the values of the children.

There is special interest in the fact that, as DIAMANT has shown, there is a not inconsiderable individual variation, inasmuch as right and left cell systems may not have the same size. The standard deviation for the differences has been calculated to give an idea of the variation here (cf. Table 5).

TABLE 5. *Difference between the cell systems on the right and left sides in cm² in children and parents.* [$\sigma \pm \varepsilon(\sigma)$ = *standard deviation* $\pm$ $\pm$ *standard error, calculated on the assumption that the right cell system is larger than the left as often as not, which implies that the mean of the differences is 0.*]

	Number	$\sigma \pm \varepsilon(\sigma)$
Children	301	$\pm 3{,}32 \pm 0{,}14$
Father	301	$\pm 3{,}51 \pm 0{,}14$
Mother	301	$\pm 3{,}37 \pm 0{,}14$

Here we have assumed that the mean is 0, i. e. that the cell system of the right ear is as often *larger* than that of the left as it is *smaller*. The standard deviation for the differences shows no significant differences between children and parents; it amounts to something over 3 cm². DIAMANT found the figure 2,03 cm²; we have thus obtained somewhat higher values.

To discover, finally, how far asymmetries are due to heredity, we have divided the parents up into those where the differences between right and left ear are greater than the median and those where they are less; we have then investigated the average values for father, mother and child (cf. Table 6). We found that, when both parents have small differences and therefore slight asymmetry, the children show some-

TABLE 6. *The average difference between the right and left cell systems in children and parents grouped according to the size of the differences between the right and left cell systems in the parents. Differences < the median have been characterized as small and differences > the median as large.* [$D \pm \varepsilon(D)$ = *mean of the differences (the signs disregarded)* ± ± *standard error of the mean.*]

Diff. between right and left in the parents	Number	Father $D \pm \varepsilon(D)$	Mother $D \pm \varepsilon(D)$	Children $D \pm \varepsilon(D)$
Both parents: small diff.	82	0,961 ± 0,065	0,851 ± 0,057	2,23 ± 0,22
Mother: large diff. Father: small »	70	0,839 ± 0,062	4,32 ± 0,28	2,45 ± 0,28
Mother: small diff. Father: large »	68	3,86 ± 0,22	0,847 ± 0,061	2,33 ± 0,29
Both parents: large diff.	81	4,33 ± 0,28	4,33 ± 0,23	2,65 ± 0,26

what less asymmetry than when both the parents have larger differences. The differences we have obtained are not great, however.

Parents who, on an average, show a difference between right and left ear of not quite 1 cm^2 have children where the average difference is $2{,}23 \pm 0{,}22$ cm^2. When the difference in the parents exceeds 4 cm^2, the difference in the children is $2{,}65 \pm 0{,}26$ cm^2. Between these two come the children in the groups (a), where the mother's difference is greater than the median and the father's less, and (b), where the reverse is the case. In other words, the asymmetry should to a certain extent depend on hereditary factors, but the latter's influence is not such as to give a strong correlation between parent and child.

The figures we have obtained, then, definitely support the statement that factors of heredity are of material importance in determining the final size of the cell system. These figures do not, however, allow a closer analysis of the hereditary course, nor is it possible to assess more definitely how far environmental factors play a part.

THE SIZE OF THE CELL SYSTEM IN TWINS.

As has already been mentioned, both monozygotic and dizygotic pairs of twins have been investigated to get an idea of the part played by environmental and hereditary factors respectively. The age distribution of the material is shown in Table 7. Though the material is not large, it should be sufficient to give an approximate idea of the

situation. As, here, we are concerned with establishing differences between twins, there seemed no very great reasons against including somewhat younger individuals also, in order to enlarge the material. Thus, 5 pairs of twins under the age of 10 have been included. Even if their cell systems cannot with certainty be considered to be fully developed, the differences can probably be regarded as stable.

TABLE 7. *Age (years completed) of the twin pairs at the examination.*

Age	Monozygotic twins	Dizygotic twins	All the twins
8	1	2	3
9	2	—	2
10	1	4	5
11	—	—	—
12	—	5	5
13	7	16	23
14	7	9	16
15	6	5	11
16	—	2	2
Unknown	—	1	1
Total	24	44	68

From a theoretical point of view, the investigation of twins is specially interesting because the differences between monozygotic twins indicate dissimilarities which are due to environment, whereas those between dizygotes are due to factors both of environment and heredity. Working from these differences and these assumptions, it is possible to calculate how great a part these respective factors play. The procedure now used in so doing has been laid down by G. DAHLBERG (1926); in a later work G. and B. DAHLBERG (1940) have gone further into the requirements of an analysis of this kind.

In this connection, it might be borne in mind that genotypical asymmetries may cause differences between monozygotic twins which are not due to heredity, and that twin material must in this respect be used with a certain reservation. Further, we have to reckon with twins living in a relatively similar environment, so that the variability due to environment may be expected to be somewhat smaller than in a population, particularly if environmental factors during the years of growth are of importance. Finally, dizygotic twins have the same parents and, therefore, a smaller range of variation in respect of hereditary factors than is to be expected in a population.

With regard to these aspects, it may first and foremost be stated that the investigation into the part played by hereditary factors in causing asymmetries in the size of the cell system has, in the first place, borne out the view that differences between right and left ear are only to a small extent due to heredity. Under such circumstances there can hardly be any great risk that genotypical asymmetries would upset the results of a comparison between twin groups. The environmental factors affecting the development and size of the cell system can hardly be other than intra-uterine, for it seems scarcely possible *a priori* that conditions during growth, such as diet, temperature, etc., should affect the cell system. The possible importance of changes in air pressure in association with a better or worse passage through the eustachian tube has been discussed, but this theory seems hardly plausible. With our present knowledge, however, it is impossible to reach definitive pronouncements on this point.

Table 8 shows, firstly, figures for the average difference in both monozygotic and dizygotic twins, secondly, figures for the standard deviation for these differences calculated from 0 (i. e. on the assumption that the cell system of the first-born twin is as often larger as smaller than that of the second). The standard deviation is calculated from these differences and gives the variation which would be obtained if separate individuals varied in the same way as monozygotic and dizygotic twins respectively round a mean. We may now begin to see that there is a significant difference between monozygotic and dizygotic twins. Dizygotic twins have, on an average, differences about twice as large as those of monozygotes, and these differences also have a significant difference for the standard deviation from 0.

TABLE 8. *Differences between the cell systems of the twins. For every individual the mean of the cell systems on the right and left sides has been calculated. At the calculations the authors have started from the assumption that the first-born twin had a larger cell system than the second-born as often as not, which implies that the mean of the differences is 0.* [$D \pm \varepsilon(D)$ = *mean of the differences (the signs disregarded)* $\pm$ *standard error of the mean.* $\sigma + \varepsilon(\sigma)$ = *standard deviation of a single individual, calculated from the differences (cf. the text).*]

	Number	D $\pm$ ε (D)	$\sigma \pm \varepsilon(\sigma)$
Monozygotic	24	2,34 $\pm$ 0,51	3,40 $\pm$ 0,49
Dizygotic	44	5,35 $\pm$ 0,51	6,32 $\pm$ 0,67
Difference between monozygotic and dizygotic	—	— 3,01 $\pm$ 0,72	— 2,92 $\pm$ 0,83

We finally see that the variation to be found in a population varying in the same way as dizygotic twins would be $\pm$ 6,32 $\pm$ 0,67. For normal material, DIAMANT found a standard deviation of about $\pm$ 5 cm^2, which in view of the standard error here implies fairly good agreement. This result indicates that the variation obtained from dizygotic twins is of about the same size as that in a population; in other words, no very great part is played by the relatively great similarity in environment during growth between dizygotic twins compared with individuals in a population. This in its turn indicates that the environmental factors which are of importance concern the foetal life. Here relatively great environmental differences obtain for dizygotic twins inasmuch as they cause comparatively great differences in stature and weight both in monozygotic and dizygotic twins (cf. DAHLBERG, 1926). We derive further support from the fact that the family material investigated here tends to present a smaller variation than the dizygotic twins. This being so, it is hardly justifiable to attribute any very great importance to the above-mentioned reservation with regard to the similar environmental conditions of the twins. On the other hand, there should always be a decreased variability in dizygotic twins as compared with a population, on account of their common derivation. In these circumstances, dizygotic twins will bring us to rather too low figures for the part played by hereditary factors, and possibly rather too high for that of environment. When dividing the variability between heredity and environment, the following equation is used:

$$\sigma_t^2 = \sigma_x^2 + \sigma_o^2$$

where σ_t is the variability obtained from dizygotic twins and referring to both heredity and environment, σ_x the standard deviation we would obtain in a population if the environment were constant and the same as the average one for twins, and if the population varied only on account of hereditary factors. Finally, σ_o is the variation calculated from monozygotic twins, which would obtain in a population if all individuals had the same genes and the environment varied in the same way as for twins. By inserting the values from Table 8 into this expression, we get 5,3 for the hereditary factors, and 3,4 for the environmental factors. Together, then, hereditary and environmental factors give a standard deviation of 6,3. In other words, if we add the value for heredity and environment direct, we get larger values than the standard deviation actually presents. This is quite simply because hereditary and environmental factors do not systematically add up with

one another; sometimes they cancel one another out, by acting in opposite directions. The result will thus be that solely hereditary factors give a variability corresponding to 84,1 % of the total variability, and solely environmental factors give a variation which is 54 % of the total one. If we compare hereditary and environmental factors, we find the former are 1,56 times more influential.

In view of this result it was natural that we should find a clear correlation between children and parents, and the results obtained by analysis of the two materials at our disposal complement one another.

As regards the problems behind this work, then, we can state that, since hereditary factors play a dominating part in determining the size of the cell system, and since the size of the cell system plays, in its turn, a dominating part in the occurrence of chronic otitis, we might expect the latter complaint to be conditioned by heredity to no mean extent.

THE RELATIVE RISK OF CHRONIC OTITIS.

It has been found that different types of chronic otitis primarily attack individuals with small cell systems. There are, however, no direct figures showing the otitis risk for individuals with cell systems of different sizes. In any case, risk figures would have only a relative value, since the general risk of infections of importance for otitis is different in different populations. A population where scarlatina and other infections are comparatively common should, of course, run greater risks of contracting different types of otitis, as also chronic otitis following an infection, than a population where such infections are not so common. The relation between the risk for groups of individuals with cell systems of different sizes should, however, be expected to be fairly constant. For this reason, figures for the relative risk of otitis are of more general interest.

Relative figures of this kind can be calculated by using the figures DIAMANT obtained in the work quoted earlier. Table 9 outlines the frequency of cell systems of different sizes in a normal material; it also shows the frequency of cell systems of different sizes in an otitis material. By placing these two numerical series in relation to one another we get risk figures which, though admittedly incorrect, ought in their turn to bear a correct relation to one another. By calculating the percentual distribution of the risks, that is to say, we arrive at some idea of the relative danger of small cell systems. Table 9 gives figures obtained in this way.

It can be seen from the table that individuals with small cell systems run a comparatively big risk of otitis. In the two age classes in the table, the grouping has been done according to means and σ. The two age groups show differences here, so that the class limits do not tally. The risk distribution obtained also presents differences, therefore. However, individuals with small cell systems are in the majority. If the limit is set at 1,5 cm^2, the group covers 72,3 % of the otitis risk. If, on the other hand, the limit for the older group is set at 0,3 cm^2, the

TABLE 9. *Percentage distribution of the risk of chronic otitis (cf. the text). The primary figures have been taken from* DIAMANT *(1940), Table 20, p. 80.*

10—30 years				30 years and above			
Size of the cell system (cm^2)	Number of cases		Percentage risk distribution	Size of the cell system (cm^2)	Number of cases		Percentage risk distribution
	Normal material	Otitis material			Normal material	Otitis material	
0 — 1,5	8	64	72,3	0 — 0,3	1	63	62,2
1,6— 4,1	18	35	17,6	0,4— 2,8	5	73	14,4
4,2— 6,8	24	21	7,9	2,9— 5,3	4	32	7,9
6,9— 9,5	63	12	1,7	5,4— 7,8	14	11	7,7
9,6—14,9	133	7	0,5	7,9—12,8	21	12	5,6
15,0—ω	110	—	0	12,9—15,3	9	2	2,2
				15,4—ω	10	—	0

group takes 62 per cent of the total risk. The series of figures agree in so far as individuals with cell systems of more than 15 cm^2 run no risk of otitis.

If we now wish to compare two groups, the figures can be used in the following way. Individuals between the ages of 10—30 years, with a cell system between 4,2 and 6,8 cm^2 — i. e. an average size of 5,5 cm^2 — run 7,9 % of the otitis risk in a population. Individuals with a cell system less than 1,4 cm^2 incur 72,3 % of the otitis risk. That is to say, the latter group is 9,2 times as liable to contract otitis as the one with a cell system of 5,5 cm^2. In other words, it can be calculated from the figures how much greater the risk is when a cell system decreases in size. There is no risk for the group with a large cell system of over 15 cm^2, and there is therefore no point in calculating figures (the result will be ∞).

A verification of the figures in the table can only be obtained by

investigating individuals in a population, following them up for a certain period, and seeing to what extent they contract chronic otitis. This would yield risk figures which could then be expected to bear that relation to one another which is given by the percentages in Table 9. Practical reasons make it very unlikely that such figures could be obtained, and under these circumstances our figures should be of a certain interest.

LITERATURE CITED.

1. ALBRECHT, W. 1931. Über Konstitutionsprobleme in der Pathogenese der Hals-, Nasen- und Ohrenkrankheiten. — Zschr. f. Hals-, Nasen- und Ohrenheilkunde, Bd. 29.
2. BAUER, J. und STEIN, C. 1925. Vererbung und Konstitution bei Ohrenkrankheiten. — Zschr. für Konstitutionslehre, Abt. 2, Bd. 10.
3. DAHLBERG, G. 1926. Twin births and twins from a hereditary point of view. — Stockholm.
4. DAHLBERG, G. und DAHLBERG, B. 1940. Über Karies und andere Zahnveränderungen bei Zwillingen. — Uppsala Läkareförenings förhandlingar. Ny följd, Bd. XLVIII, 5—6.
5. DIAMANT, M. 1940. Otitis and pneumatisation of the mastoid bone. — Acta Oto-Laryngologica, Suppl. XLI.
6. DILLON, I. G. and GOUREVITCH, I. B. 1936. Research on the pneumatisation of the nasal accessory sinuses and of the mastoid processes and on the shape and dimensions of sella turcica in twins. — The American Journal of Roentgenology, Vol. 35.
7. LEICHER, H. 1928. Die Vererbung anatomischer Variationen der Nase, ihrer Nebenhöhlen und des Gehörorgans. — München.
8. OKASAKI, M. 1935. Röntgenologische Studien über die Bedeutung der Erbfaktoren bei der Pneumatisation der Warzenfortsätze. — Jahresvers. d. Japan. Oto-Rhino-Laryngol. Ges., Niigata. (Cit.: Zentralblatt für Hals-, Nasen- und Ohrenheilkunde, Bd. 29.)
9. PAULSEN, J. 1925. Beobachtungen an eineiigen Zwillingen. — Arkiv für Rassen- und Gesellschaftsbiologie, Bd. 17.
10. SCHWARZ, M. 1929. Die Bedeutung der hereditären Anlage für die Pneumatisation der Warzenfortsätze und der Nasennebenhöhlen. — Arkiv für Ohren-, Nasen- und Kehlkopfheilkunde, Bd. 123.
11. — 1935. Ererbte Taubheit. — Leipzig.
12. — 1937. Der Erbfaktor bei der Pneumatisation des Warzenfortsatzes. — Der Erbarzt, Bd. IV.
13. STEIN, C. 1917. Gehörorgan und Konstitution. — Zschr. für Ohrenheilkunde und für die Krankheiten der Luftwege, Bd. 76.
14. WEITZ, W. 1925. Studien an eineiigen Zwillingen. — Zschr. für klinische Medizin, Bd. 101.

CYTOLOGICAL STUDIES OF EXTRA FRAGMENT CHROMOSOMES IN RYE

II. TRANSMISSION AND MULTIPLICATION OF STANDARD FRAGMENTS AND ISO-FRAGMENTS

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IN the offspring of a single rye plant with two additional small chromosomes observed in the variety »Östgöta Gråråg» (MÜNTZING and PRAKKEN, 1941) the effects of a varying number of such extra »fragment chromosomes» were studied (MÜNTZING, 1943). Fertility was found to be more influenced than the vegetative development, but also with regard to vigour a definite influence was demonstrated. Thus, the fragment chromosomes are not inert as was supposed by some previous workers. However, considering the size of the fragments and the strong effects of extra chromosomes in trisomic plants, it is probable that the fragments are subinert, having a lower proportion of active genes than ordinary chromosomes.

As a rule extra fragment chromosomes of the usual type (the »standard fragments») are stable, but in rare cases their centromere may misdivide (MÜNTZING, 1944). The two products of misdivision, corresponding to the short and long arms of the standard fragments, were recovered in the shape of iso-fragments with median centromeres (l. c.). Thus, in addition to plants with standard fragments (»s. f.») plants with large and small iso-fragments (»iso-f.») are now available in the material of »Östgöta Gråråg».

The present report primarily deals with the transmission of the extra fragment chromosomes in various cross combinations. These studies were undertaken firstly in order to compare the behaviour of the iso-fragments and the standard fragments, secondly to elucidate the peculiar fragment frequencies in the offspring observed by LEWITSKY, MELNIKOV and TITOVA (1932) and by the present writer (1943, pp. 93—94). In crosses between parents with $2n = 14$ and $2n = 14 + 2$ fragments most of the hybrids have either $2n = 14$ or $14 + 2$ fragments and not $14 + 1$ fragment as might be expected. The probable cytological cause of this peculiar result was observed by HASEGAWA

(1934). According to this author, the extra fragment chromosome shows a rather irregular behaviour at anaphase of the first pollen mitosis. Its two halves often do not separate, and in most cases they are included in the generative nucleus. A similar process must be assumed to occur on the female side as well.

The latter part of the present paper deals with the structure, pairing properties and transmission of extra fragment chromosomes found in another rye variety, »Vasa II».

I. TRANSMISSION OF THE STANDARD FRAGMENTS IN »ÖSTGÖTA GRÅRÅG».

1. CROSSES BETWEEN PLANTS WITH $2n = 14$ AND PLANTS WITH $2n = 14 + 1$ STANDARD FRAGMENT.

From the cross $14\ ♀ \times (14 + 1$ s. f.$)\ ♂$ only 3 plants were obtained. Two of them had $2n = 14$ and one plant $2n = 14 + 2$ s. f. In the reciprocal cross $(14 + 1$ s. f.$)\ ♀ \times 14\ ♂$ a total of 69 F_1 plants were obtained from 9 mother plants. Of these F_1 plants 65 had $2n = 14$, the remaining 4 plants, occurring in 4 different families, had $2n = 14 + 2$ s. f. Not a single plant had $2n = 14 + 1$ s. f. Thus, all the F_1 individuals carrying standard fragments had them in double number. This demonstrates that non-disjunction of the fragment must have occurred on the male as well as the female side of the parent with $2n = 14 + 1$ s. f.

The proportion of F_1 plants with fragments is surprisingly low, at least in the cross $(14 + 1$ s. f.$) \times 14$, the percentage value in this case being 5,8. This low value may be caused by a rather pronounced elimination of the single fragment at various stages of meiosis. Though a detailed study of the meiotic elimination has not yet been made, it does not seem probable that this elimination is quite sufficient to account for the low proportion of daughter plants with standard fragments. It is possible that the fragments are also to some extent eliminated during the postmeiotic divisions in the embryo-sac. During this stage, just as in the pollen mitosis, there must be a tendency to non-disjunction. If the process of non-disjunction is not regular, the result will probably be a certain degree of elimination. Unfortunately the number of plants obtained from the cross $14 \times (14 + 1$ s. f.$)$ was too low to allow any safe conclusions as to the rate of elimination in the pollen. Crosses of this kind will be repeated.

2. INTERCROSSES BETWEEN PLANTS WITH $2n = 14 + 1$ s. f.

In 1944 seven plants with $2n = 14 + 1$ s. f. were put together as a small separation in a greenhouse. From the plants thus intercrossed five progenies were raised, comprising a total of 52 plants. Forty-five of the plants had $2n = 14$, 2 plants $2n = 14 + 1$ s. f. and 5 plants $2n = 14 + 2$ s. f. Thus, the percentage of plants with fragments amounted to 13,5. In the crosses $14 \times (14 + 1$ s. f.) and reciprocally, discussed above, 5 plants of 72, i. e. 6,9 per cent, were found to contain fragments. Supposing the proportion of gametes with fragments to be 6,9 per cent on the male as well as the female side, intercrosses between plants with $14 + 1$ s. f. should give progenies containing 86,7 per cent of plants with no fragments and 13,3 per cent with fragments. As the ratio actually found was 86,5 : 13,5, expectation and observation show a very good conformity.

3. CROSSES BETWEEN PLANTS WITH $2n = 14$ AND PLANTS WITH $2n = 14 + 2$ s. f.

Six different progenies were raised from crosses of the type $(14 + 2$ s. f.) ♀ $\times$ 14 ♂. All these progenies showed about the same distribution, and hence only the following total result will be given:

Number of standard fragments:	0	1	2	3	4
Number of plants:	62	5	46	—	2

Thus, most of the 115 plants had either 0 or 2 fragments, the former class being the largest. The number to be expected from the cross, $2n = 14 + 1$ s. f., is only represented by 5 plants and finally there were 2 individuals with 4 fragments.

The values obtained show again that there is a process of non-disjunction on the female side. The ovules carrying an even number of fragments (2 or 4) were about ten times as frequent as those carrying one fragment.

In the previous cross, $(14 + 1$ s. f.) $\times$ 14, the fragments were only transmitted to 5,8 per cent of the offspring, and it was suggested that part of this elimination might occur during the postmeiotic divisions. The ratios obtained in the present cross demonstrate that meiotic elimination also plays an important rôle and accounts for part of the difference between the crosses $(14 + 1$ f.) $\times$ 14 and $(14 + 2$ f.) $\times$ 14. This is evident from the following calculation. If there were no meiotic elimination in a plant with one fragment, 50 per cent of the macro-

spores would receive 1 fragment and 50 per cent no fragment. Owing to postmeiotic elimination the former category would be divided into 44,2 per cent giving egg cells without fragments and 5,8 per cent with fragments. This would account for the proportion of plants with fragments actually observed. If there were no meiotic elimination in a plant with two fragments, all macrospores would receive one fragment. Assuming the same degree of postmeiotic elimination as in plants with one fragment, the proportion of egg cells with fragments would evidently be twice as high or 11,6 per cent. The proportion actually observed was much higher, the percentage value being 46,1. The cause of the difference is certainly that in plants with two fragments these fragments are often paired to form a small bivalent. The regular disjunction in this bivalent will considerably increase the proportion of macrospores and offspring with fragments.

Though sufficient to explain the difference in the rate of elimination between plants with one and two fragments, the degree of pairing between the two fragments in plants with $2n = 14 + 2$ s. f. shows a certain variation. In the plant with $14 + 2$ s. f. first detected the fragments were found to be unpaired in about two thirds of the p. m. c. (MÜNTZING and PRAKKEN, 1941, p. 279). In 8 other plants with $2n = 14 + 2$ s. f. the percentage of p. m. c. with unpaired fragments was found to range from 53 to 86 with a total average of 61,8.

The rather frequent lack of pairing between the two fragments accounts for the production of plants with $14 + 4$ s. f. in the crosses $(14 + 2$ s. f.$) \times 14$. In some cases both fragments may evidently be included in the same macrospore and both may undergo non-disjunction and pass to the egg cell.

In the reciprocal combination, $14\ ♀ \times (14 + 2$ s. f.$)\ ♂$, data are available from five progenies, showing about the same distributions. The following total values were obtained:

Number of fragments:	0	1	2	3	4
Number of plants:	22	9	13	—	2

The percentage of plants with fragments is 52,2 and, thus, slightly but not significantly higher than in the reciprocal cross (46,1 %). Another difference is more remarkable, viz. the rather high proportion of plants with one extra fragment. In the present cross combination the number of plants with one and two extra fragments is 9 and 13 respectively. In the reciprocal cross the corresponding numbers were

5 and 46. A χ^2 test of the distributions 9 : 13 and 5 : 46 showed them to be significantly different, χ^2 being 9,594 and P intermediate between 0,01 and 0,001. Thus, the mechanism of non-disjunction seems to have been more effective on the female side than in the pollen.

Of the 9 plants with one extra fragment 6 had a typical standard fragment, whereas in 3 plants (of the same progeny) it was a small iso-fragment of the kind previously described (MÜNTZING, 1944).

In the experiments of LEWITSKY, MELNIKOV and TITOVA (1932) the elimination of the extra fragments is evidently less pronounced than in the present case. In the cross (14 + 2 s. f.) × 14 these authors obtained 10 plants with 2 extra fragments, and 4 plants without fragments. In the reciprocal combination the result was 2 plants with no fragments, 2 plants with one fragment, 29 plants with two fragments and 1 plant with three fragments. This indicates a higher degree of pairing between the fragments and a more regular distribution than in my material of »Östgöta Gråråg». Otherwise the results are rather similar.

4. CROSSES OF THE TYPE (14 + 2 s. f.) × (14 + 2 s. f.) AND ISOLATION OF PLANTS WITH 14 + 2 s. f.

Crosses between plants with 2n = 14 + 2 s. f. were undertaken repeatedly in order to preserve the material containing extra fragment chromosomes. Crosses of the same kind also occurred by open pollination in isolation groups, exclusively composed of plants with 2 standard fragments. Chromosome counts were undertaken in 10 different progenies, the following total result being obtained:

Number of extra fragment chromosomes:	0	1	2	3	4	5	n	M
Number of plants:	26	8	60	8	23	2	127	2,00

The average number of standard fragments in this series is 2,00 and, thus, crosses between plants with 2n = 14 + 2 s. f. give progenies with about the same number of fragments as in the parent types. The average number of standard fragments in the ten progenies, composing the total series given above, were the following: 1,67; 2,31; 2,62; 0,61; 1,92; 2,30; 2,14; 2,25; 1,70 and 2,40. Six of these values are above 2,00, four values are lower.

Fifteen plants of the same cross combination were studied by LEWITSKY and his co-workers (l. c.). Four of these plants had 2 extra fragment chromosomes, one plant 3 fragments and ten plants 4 frag-

ments. This distribution differs from my series by a complete absence of plants without fragments and by a very clear average increase in fragment number. The cause of this difference is probably again a better pairing and less elimination in the Russian material. This conclusion is supported by data from the other rye variety »Vasa II» given below.

In the material of »Östgöta Gråråg» now under consideration the maintenance of an average of 2,00 standard fragments is evidently due to an equilibrium between two opposing forces, viz. elimination of unpaired fragments and the ability of such fragments to increase in number by non-disjunction. The latter ability is again reflected by the great preponderance of plants with an even number of fragments. In the classes with 2 and 4 fragments there is a total of 83 plants, in the classes with 1, 3 and 5 fragments only 18 plants.

The frequencies of standard fragments in the crosses $(14 + 2\,\text{s.f.}) \times (14 + 2\,\text{s.f.})$ may be compared with the values obtained in the crosses $14 \times (14 + 2\,\text{s.f.})$ and reciprocally. According to these values (p. 459), plants with $2n = 14 + 2$ s. f. produce pollen grains which in 47,8 per cent of the cases are free from fragments, the other 52,2 per cent carrying a variable number of fragments. On the female side the corresponding values are 53,9 per cent without fragments and 46,1 per cent with fragments. If these values are combined, the expectation for intercrosses of plants with $14 + 2$ s. f. will be 74,2 per cent of the plants with fragments. The proportion actually obtained was 79,5 % (101 plants of 127). Thus, there is a quite good conformity between observation and expectation.

As might be expected, the offspring of isolated plants with $14 + 2$ s. f. gave a chromosomal variation of just the same type as the intercrosses of such plants. After isolation of plants with $14 + 2$ s. f. five progenies were raised, the following total result being obtained:

Number of extra fragment chromosomes:	0	1	2	3	4	n	M
Number of plants:	11	1	18	7	9	46	2,04

According to these figures, 76,1 per cent of the plants had fragments. This corresponds very well with the expected value (74,2) and the value obtained in the intercrosses (79,5). The average number of fragments is also about the same as in the intercross material.

Finally it may be mentioned that in three cases progenies were raised from open-pollinated plants with $14 + 2$ s. f. A total of 74

daughter plants were examined and found to have the following number of standard fragments:

Number of fragments:	0	1	2	3	4	n	M
Number of plants:	27	6	29	6	6	74	1,43

In this case the average number of fragments (1,43) and the percentage of plants with fragments (63,5) are lower than in the previous progenies of plants with 14 + 2 s. f. This is not unexpected, as the mother individuals had been surrounded not only by plants with a variable number of fragments but also by plants with 2n = 14.

Before leaving the progenies of plants with two standard fragments it may be mentioned that in three cases new fragment types have arisen. These novelties were described previously (MÜNTZING, 1944). Thus, of the eight plants with one fragment obtained from the intercrosses (14 + 2 s. f.) × (14 + 2 s. f.) one plant had a small iso-fragment and another one a large iso-fragment. Further, one of the plants with three fragments derived from the same crosses contained one small iso-fragment plus two standard fragments.

5. PROGENIES OF PLANTS WITH 14 + 4 s. f. AND 14 + 6 s. f.

Some offspring were raised from intercrosses, isolation and open pollination of mother plants with 14 + 4 s. f. These progenies showed the following chromosomal variation:

Number of fragments:	0	1	2	3	4	5	6	n	M
(14 + 4 s. f.) × (14 + 4 s. f.):	2	1	4	—	4	—	4	15	3,27
14 + 4 s. f., isolation:	1	—	2	1	5	—	4	13	3,92
14 + 4 s. f., open pollination:	8	4	14	1	12	1	2	42	2,38

According to these values, the plants with 14 + 4 s. f. are not quite able to maintain an average number of 4 fragments in the offspring from intercrosses or isolation. In the progenies after open pollination there is, of course, a still more pronounced tendency towards a lower number of fragments. As in the progenies of plants with 14 + 2 s. f., there is an obvious preponderance of plants with an even number of fragments. This was also observed by LEWITSKY, MELNIKOV and TITOVA who obtained 8 plants from a cross between two individuals having 4 fragments. Four of the daughter plants had 4 fragments, two 6 fragments, one 2 fragments and one 0 fragment. Thus, the same

mechanism of non-disjunction met with in the plants with one or two standard fragments is also at work in the plants with 14 + 4 s. f.

As the plants with 6 extra fragment chromosomes so far obtained were rather poor and sterile, seeds could only be obtained after open pollination. From such kernels, harvested on 4 different mother plants, at total of 37 daughter plants were obtained. The number of fragments in these plants was found to vary as follows:

Number of fragments:	0	1	2	3	4	5	6	n	M
Number of plants:	7	—	12	3	10	3	2	37	2,70

There is evidently a very marked average decrease in fragment number, and several plants without fragments were also produced. This demonstrates that in the ovules all six fragments may sometimes be eliminated during meiosis or the postmeiotic divisions.

II. TRANSMISSION OF THE ISO-FRAGMENTS IN »ÖSTGÖTA GRÅRÅG».

In 1944 seven plants having $2n = 14 + 1$ small iso-fragment were left for intercrossing in a greenhouse separation. At the same time artificial crosses were made in both directions between these plants and plants without fragments. Exactly the same procedure was undertaken with 5 plants containing one large iso-fragment. The seeds obtained from these crosses and intercrosses were germinated last autumn. The root tips of the seedlings were examined with the following results.

1. CROSSES BETWEEN PLANTS WITH $2n = 14$ AND $2n = 14 + 1$ SMALL ISO-FRAGMENT.

These cross combinations as well as all other crosses described in the present paper succeeded without the slightest difficulty. Five different mother plants, involved in the crosses $14 \times (14 + 1$ small iso-f.), gave a total of 67 daughter plants; 62 of these plants were free from fragments whereas the remaining 5 plants had one small iso-fragment.

In the reciprocal crosses, $(14 + 1$ small iso-f.$) \times 14$, a similar result was obtained. Six different mother plants gave 68 daughter plants. Sixty-five of these had no fragments, the remaining 3 plants having one small iso-fragment.

The results obtained demonstrate a rather high degree of elimination, the percentage of plants with fragments being as low as 7,5

per cent in the crosses first mentioned and 4,4 per cent in the reciprocal combination. Combining both crosses combinations the total percentage is 5,9. This value, however, is rather close to the values obtained in the crosses 14 × (14 + 1 standard fragment) and reciprocally (cf. p. 458). Thus, in this respect the small iso-fragment behaves like the standard fragment. However, in contrast to the standard fragment the small iso-fragment is not capable of performing the peculiar trick of postmeiotic non-disjunction. Therefore, in the plants with fragments there was only one fragment, not two as in the corresponding crosses with the standard fragments.

2. INTERCROSSES BETWEEN PLANTS WITH 2n = 14 + 1 SMALL ISO-FRAGMENT.

Five progenies were raised from the separated group composed of plants with one small iso-fragment in addition to the 14 normal chromosomes. The different progenies behaved in the same way and comprised a total of 57 plants without fragments and 7 plants with one small iso-fragment. Thus, also in these progenies the small iso-fragment showed itself incapable of multiplication by means of non-disjunction.

In the crosses 14 × (14 + 1 small iso-f.) and reciprocally the percentage of plants with fragments was found to be 7,5 and 4,4. On the basis of these values the percentage to be expected from intercrosses between plants with 14 + 1 small iso-f. will be 11,6. More accurately, 11,24 per cent should have 1 fragment and 0,33 per cent 2 fragments. The observed percentage was 10,9, all these plants having 1 fragment. In a larger material plants with 2 fragments probably would have appeared. At any rate there is a very good conformity between observation and expectation.

3. CROSSES BETWEEN PLANTS WITH 2n = 14 AND 2n = 14 + 1 LARGE ISO-FRAGMENT.

A total of 135 plants were raised from artificial crosses between plants with 2n = 14 and plants with one additional large iso-fragment. In 4 progenies, representing the cross 14 × (14 + 1 large iso-f.), 72 plants were obtained. 64 of these plants had no fragments, the remaining 8 plants (11,1 per cent) all had 2n = 14 + 2 large iso-fragments. In the reciprocal combination 4 progenies were also available. They were found to comprise 61 plants without fragments and 2 plants (3,2 per cent) having 2n = 14 + 2 large iso-fragments.

In the first place the results obtained demonstrate that the large iso-fragment, just as the standard fragment, is capable of a regular postmeiotic non-disjunction. Not a single plant in the offspring had one large iso-fragment, this chromosome always being duplicated. However, plants with 14 + 1 large iso-f. do arise in other cases. Three of the plants of this kind available in 1944 had appeared in the offspring of open-pollinated plants with 2n = 14 + 1 small iso-f. The large iso-fragment was certainly derived from the pollen of plants with large iso-fragments growing close by. A few plants with 14 + 1 large iso-f. also appeared in the offspring of the crossing group described below.

4. INTERCROSSES BETWEEN PLANTS WITH LARGE ISO-FRAGMENTS.

In order to preserve a rye material containing large iso-fragments all the 5 plants with such fragments available in 1944 were left for intercrossing in a greenhouse separation. Three of these plants had 2n = 14 + 1 large iso-f., one plant had 2n = 14 + 2 large iso-f. + 1 standard-f., and the fifth plant 2n = 14 + 2 large iso-f. + 2 standard-f. The presence of the latter two plants was disturbing from a theoretical point of view, as the effect of crosses between plants with only one large iso-f. could not be studied. However, a brief report of the plants obtained is still of interest.

A. THE OFFSPRING OF THE PLANTS WITH 2n = 14 + 1 LARGE ISO-F.

The three mother plants of this kind behaved in a similar way and gave a total of 41 daughter plants showing the following chromosomal variation:

Chromosomal constitution:	2n = 14	14 + 1 large iso-f.	14 + 1 s. f.	14 + 2 large iso-f.	14 + 2 large iso-f. + 1 s. f.	14 + 4 large iso-f.
Number of plants:	23	4	1	11	1	1

41,5 per cent of these plants have large iso-fragments. This is a higher proportion than would be expected if only pollen of the plants with 14 + 1 large iso-f. had been available. The higher proportion is certainly due to the presence in this crossing group of plants containing 2 large iso-fragments. A high proportion of the pollen grains of these plants will certainly carry large iso-fragments and thus increase the frequency of plants with such fragments in the offspring of the mother plants with 2n = 14 + 1 large iso-f. It should be observed that in

13 plants of 17 the large iso-fragment was present in an even number (2 fragments in 12 plants, 4 fragments in 1 plant). This confirms the previous evidence that as a rule the large iso-fragment, just as the standard fragment, undergoes a postmeiotic non-disjunction. Only 4 daughter plants had 2n = 14 + 1 large iso-f. like the mother individuals.

B. THE OFFSPRING OF THE PLANTS WITH TWO LARGE ISO-FRAGMENTS.

As mentioned above, the two mother plants in question had both 2 large iso-fragments and in addition 1 and 2 standard fragments respectively. In the offspring the following variation was observed:

Mother plant: 14 + 2 large iso-f. + 1 s. f.

Offspring:

2n = 14: 2 plants
» = 14 + 2 s. f.: 3 »
» = 14 + 2 large iso-f.: 6 »
» = 14 + 3 » » : 1 plant
» = 14 + 4 » » : 1 »
» = 14 + 2 s. f. + 1 large iso-f.: 1 plant

Mother plant: 14 + 2 large iso-f. + 2 s. f.

Offspring:

2n = 14: 2 plants
» = 14 + 2 large iso-f.: 4 »
» = 14 + 2 s. f.: 6 »
» = 14 + 3 » : 1 plant
» = 14 + 4 » : 1 »
» = 14 + 2 s. f. + 2 large iso-f.: 1 plant
» = 14 + 4 s. f. + 4 s. f. + 1 large iso-f.: 1 plant

In both progenies there is a rather high proportion of plants with large iso-fragments. This is certainly due to the presence of pairs of such fragments in the mother plants. Standard fragments are also frequent, especially in the progeny of the mother plant having a pair of standard fragments. In most cases standard fragments as well as large iso-fragments are evidently present in even numbers.

III. STUDIES ON EXTRA FRAGMENT CHROMOSOMES IN THE VARIETY »VASA II».

During colchicine work with plants of the rye variety »Vasa II» a single individual with two extra chromosomes was detected. In aceto-carmine smears a rather regular pairing of these extra chromosomes was observed and they also seemed to be larger than the standard fragments in »Östgöta Gråråg» (cf. MÜNTZING, 1943, p. 108).

As, further, the variety »Vasa II» had not previously been included in my material and the seed sown was commercial seed, it seemed highly probable that the extra chromosomes observed were quite different in origin from those previously studied in »Östgöta Gråråg».

1. TRANSMISSION OF THE EXTRA CHROMOSOMES.

From the original plant of »Vasa II» having $2n = 14 + 2$ f. offspring were raised after open pollination. A total of 32 plants were obtained and found to represent the following chromosomal variation:

Number of fragment chromosomes:	0	1	2	3	4
Number of plants:	5	1	25	—	1

Most of the surrounding plants of »Vasa II» had $2n = 14$ or $2n = 28$, due to colchicine doubling. Thus, the progeny after open pollination must really represent a cross of the type $(14 + 2$ f.$) \times 14$. The result of this cross shows that the fragment in »Vasa II» has the same capability of non-disjunction as the fragment in »Östgöta Gråråg». It differs from this fragment, however, by a much higher rate of transmission to the offspring. In the case of »Östgöta Gråråg» the first progeny, exactly comparable to the one now under consideration, was composed of 10 plants without fragments and 10 plants with fragments. In the comparable crosses $(14 + 2$ s. f.$) \times 14$ reported above (p. 459) less than half of the daugther plants had fragments. In the present case, on the contrary, as many as 84 per cent of the plants in the first progeny had fragments.

The same behaviour was repeated in the next generation. Four progenies of plants with $2n = 14 + 2$ f. were raised after open pollination and one progeny after isolation. The latter one was composed of 1 plant with one fragment, 6 plants with two fragments and 2 plants with four fragments. Not a single plant was free from fragments, and the average fragment number was higher than in the mother plant. The four progenies after open pollination gave the following total result:

Number of fragments:	0	1	2	3	4	n	M
Number of plants:	4	—	24	—	24	52	2,77

The almost complete absence of plants with $2n = 14$ is striking, and an average increase in fragment number is quite obvious. The

preponderance of plants with an even number of fragments is also very clear. This series may be compared with the series representing progenies of open-pollinated, isolated and intercrossed (14 + 2 s. f.)-plants in »Östgöta Gråråg» (pp. 461—463). The difference is very striking and demonstrates that in »Vasa II» the fragments are transmitted to the offspring in a much higher degree than in »Östgöta Gråråg».

2. THE DEGREE OF FRAGMENT PAIRING AT MEIOSIS.

The very first meiotic observations indicated a higher degree of pairing of the fragments in »Vasa II» than in »Östgöta Gråråg», and this was verified by further studies.

In »Östgöta Gråråg» the original plant with $2n = 14 + 2$ f. was found to have the fragments unpaired in 72 per cent of 488 cells examined. Eight plants with 14 + 2 s. f. examined in later generations were found to have the following percentages of cells with unpaired fragments: 76, 61, 48, 45, 53, 86, 60, 65. Each value is based on counts of about 100 cells. The total average is 61,8 which is somewhat lower than the value of the original plant (72).

Counts of the same kind were undertaken in the »Vasa II» material. In this case the original plant was found to have 12 per cent of cells with unpaired fragments. In seven daughter plants the corresponding values were found to be 7, 30, 24, 2, 2, 8 and 5, the average of these values being 11,1 per cent. Thus, though there is some variation between plants, it is quite obvious that in the »Vasa II» material there is a significantly higher degree of pairing between the extra fragment chromosomes than in »Östgöta Gråråg». This explains the fact that the frequency of plants with fragments and the average number of fragments in the offspring of 14 + 2 f. plants are much higher in »Vasa II» than in the other variety.

3. SIZE AND MORPHOLOGY OF THE FRAGMENTS IN »VASA II» AND »ÖSTGÖTA GRÅRÅG».

The first meiotic observations indicated that the fragments were larger in »Vasa II» than in »Östgöta Gråråg», and this probable size difference might perhaps explain the different degree of pairing. A size difference was, indeed, established by the measurements given below but was found to be much smaller than expected. The general appearance of the »Vasa II» fragments is the same as that of the standard fragments in »Östgöta Gråråg» (MÜNTZING, 1944, Figs. 6—7). Thus,

in mitosis they may easily be distinguished from the other chromosomes by a smaller size and by the subterminal position of the centromere.

Measurements were performed in mitosis as well as meiosis. In the root tips the length of the fragment chromosomes was found to be as follows:

Fragment length:	13	14	15	16	17	18	19	20	21	22 units	n	M
»Östgöta Gråråg» (12 plants):	1	4	4	14	19	15	4				61	17,25
»Vasa II» (the original plant):					2	8	5	6	1		22	19,32
» » (10 daughter plants):			2	1	3	16	11	9	5		47	19,20
», total :			2	1	5	24	16	15	6		69	19,24

Each unit in the table corresponds to 0,23 μ. The average fragment length in »Vasa II» (19,24) is evidently greater than in »Östgöta Gråråg» (17,25), the former value being 11,5 per cent higher than the latter one. The significance of the difference was tested with an analysis of variance. This gave a v^2 of 71,43 and a P smaller than 0,001.

TABLE 1. *Average fragment diameter at I—M.*

	4,5	5,0	5,5	6,0	6,5	7,0	7,5	8,0 units	n	M
»Vasa II», plant 1					5	5	7	3	20	6,95
» » 2			1	—	1	4	10	4	20	7,10
» » 3				1	6	6	5	2	20	6,78
» » 4				2	6	3	8	1	20	6,75
» » 5					6	6	6	2	20	6,85
» » 6					11	4	5		20	6,60
»Vasa II», total			1		3	35	28	41	12	120	6,84
»Östgöta Gråråg», plant 1					2	6	8	4	20	6,60
» » » 2			1		4	7	3	5	20	6,43
» » » 3			2		1	5	10	2	20	6,48
» » » 4			1		1	6	9	3	20	6,55
» » » 5			3		2	8	6	1	20	6,25
» » » 6		1	1		9	8	1		20	5,93
»Östgöta Gråråg», total		1	8		19	40	37	15	120	6,37

At meiosis the size of unpaired fragments in plants with $2n = 14 + 2$ s. f. was measured at first metaphase, the average of length and breadth being determined. Measurements were undertaken in 6 plants of each variety. As is evident from Table 1, the average fragment diameter in the plants of »Vasa II» ranged from 6,60 to 7,10

with a total average of 6,84. In the plants of »Östgöta Gråråg» the corresponding values ranged from 5,93 to 6,60 with a total average of 6,37. The significance of the difference between the total average values, 6,84 and 6,37, was tested with an analysis of variance. The v^2 value in this case was found to be 42,70, giving a P smaller than 0,001. Thus, from measurements of the fragments at mitosis as well as meiosis it is evident that the standard fragment in »Östgöta Gråråg» is slightly but significantly smaller than the corresponding fragment in »Vasa II».

IV. DISCUSSION.

The striking difference in pairing capacity between the fragment chromosomes in »Östgöta Gråråg» and »Vasa II» may be due either to a different structure of the fragments in the two varieties or to genotypical control. Measurements at mitosis and meiosis revealed, indeed, that the fragments in »Vasa II» were significantly larger than in the other variety. This size difference, however, is rather slight and seems to be quite insufficient to explain the very different degree of pairing in a chromosome-mechanical way. As is well-known the chiasma frequency is otherwise in many cases roughly proportional to chromosome length (cf. STONE and MATHER, 1932). Under such circumstances it seems most probable that the different degree of pairing and also the small size difference is genotypically controlled, the genetic background in »Vasa II» differing in some way from that in the other variety. This problem may be further elucidated by cytological studies in hybrids between the two varieties.

The assumption that the different degree of pairing is genotypically controlled is further strengthened by the fact that the general morphology of the extra chromosomes in »Vasa II» is quite the same as in »Östgöta Gråråg». In both varieties the fragment chromosomes are smaller than the other chromosomes and have a subterminal centromere. The long arm of the fragments is about 5 times as long as the short, headlike arm. It is still more interesting that the same morphology has been described by LEWITSKY (1931) and HASEGAWA (1934) for the extra fragment chromosomes occurring in Russian and Japanese rye populations. In a previous paper (1943) I expressed the view that the extra chromosomes sometimes met with in rye are of various kinds, that they are frequently produced anew from plants with 14 chromosomes, and that strains with extra chromosomes are short-lived. This view was based on the frequent occurrence of fragmentation in ordinary

population plants of rye, on the size differences between the fragments in a 16-chromosome plant examined by DARLINGTON (1933), and on the different degrees of fragment pairing observed by HASEGAWA, DARLINGTON and myself. However, considering all data now available, it seems more plausible that there is an old and widely distributed standard type of extra chromosomes in rye, and that the deviating fragment types noticed are only occasional and more or less ephemeral deviations from this standard type.

A very striking property of the standard fragment is the ability of numerical increase by means of non-disjunction. This process was first observed by HASEGAWA (1934) in the first pollen mitosis. At this division the two chromatids of the fragment chromosome usually pass to the generative nucleus and, thus, male gametes with $7 + 2$ s. f. are produced. Sometimes this non-disjunction is unsuccessful, both chromatids are eliminated, and the result is pollen grains with 7 ordinary chromosomes and no fragments. Only in rare cases was the fragment chromosome found to divide regularly. In *Sorghum purpureo-sericeum* a similar non-disjunction of extra »B-chromosomes» was observed by DARLINGTON and THOMAS (1941). In this case the process occurs at a second division of the vegetative nucleus in the pollen grain, but the final effect is the same, a multiplication of the extra chromosomes.

The experimental data reported above definitely demonstrate the occurrence of non-disjunction on the male as well as the female side of my rye plants, the rate of non-disjunction being about the same on both sides or possibly still more marked in the ovules. Just as in the p. m. c., non-disjunction during meiosis in the ovules cannot account for the rarity of egg cells with $7 + 1$ s. f. In plants with $14 + 2$ s. f. macrospores with $7 + 2$ s. f. may of course sometimes be formed. At I—M in the p. m. c. and probably also in the e. m. c. the fragments are often unpaired (especially in »Östgöta Gråråg») and both may be included in the same interphase nucleus. However, the great majority of the macrospores will receive $7 + 1$ s. f. or $7 + 0$ s. f. owing to elimination. Hence, in most cases, at least, the egg cells with $7 + 2$ s. f. must be produced by a postmeiotic non-disjunction.

If no meiotic elimination occurred each macrospore would receive 1 standard fragment. Postmeiotic non-disjunction in such a case would give 50 per cent of egg cells with 2 fragments, if the process of non-disjunction is at random, and a higher percentage of egg cells with 2 fragments in case of a directed non-disjunction favouring the egg cell. In the crosses $(14 + 2$ s. f.$) \times 14$ 46,1 per cent of the egg cells were

found to carry fragments, most of them having 2 fragments. This would seem to be in accordance with the 50 per cent expected from random postmeiotic non-disjunction. However, from the comparison between the results of the crosses $(14 + 1 \text{ s. f.}) \times 14$ and $(14 + + 2 \text{ s. f.}) \times 14$ it could be concluded that a good deal of meiotic elimination occurs in plants with 1 standard fragment. Now, in plants with 2 standard fragments these fragments are only paired in about 40 per cent of the cells. Thus, in more than half of the cells the fragments are unpaired, and this will cause a rather high degree of elimination. Consequently a great many macrospores will not receive any fragments. As, nevertheless, almost 50 per cent of the egg cells carried fragments and generally in double number, this must imply that the process of non-disjunction is directed, i. e. the two fragments more frequently pass to the egg cell than to other cells in the female gametophyte. It remains to be settled at which of the divisions in the embryosac this non-disjunction occurs.

Further evidence of a directed non-disjunction on the female side was obtained from the very high frequency of plants with $14 + 2$ s. f. in the offspring of open-pollinated plants with $14 + 2$ s. f. in »Vasa II». The single original plant with $14 + 2$ s. f. was surrounded by ordinary plants without fragments and, thus, the offspring of this plant may be considered to represent a cross of the type $(14 + 2 \text{ s. f.}) \times 14$. As reported above (p. 468), not less than 84 per cent of these plants had fragments. Random non-disjunction would give a maximum of 50 per cent of plants with fragments or rather a slightly lower value owing to some degree of meiotic elimination. The difference between the observed ratio 27 : 5 and the expected one 16 : 16 is significant ($\chi^2 = 15{,}12$).

As mentioned above, preliminary observations of HASEGAWA (1934) indicated that non-disjunction in the pollen mitosis is also directed. In 13 cells of 16 the two fragment chromosomes were included in the generative nucleus. The experimental results now reported point in the same direction. In spite of the rather pronounced meiotic elimination in plants with $14 + 2$ s. f. the cross combination $14 \times (14 + 2 \text{ s. f.})$ gave an offspring in which as many as 52,2 per cent of the plants had fragments. Several of these plants, however, had only one fragment, and as pointed out above (p. 461) the mechanism of non-disjunction in this material seems to be more effective on the female side than in the pollen.

The occurrence of a directed non-disjunction on the male as well as the female side strongly tends to increase the number of fragments

in the offspring. This tendency is counteracted by the meiotic elimination of the fragments. This elimination was found to be very marked in plants with a single extra fragment. According to the data gathered for plants with 14 + 1 s. f., only about 6,9 per cent of the gametes transmit fragments to the offspring.

In plants of »Östgöta Gråråg» with 14 + 2 s. f. the occurrence of fragment elimination was also obvious, though the rate of this elimination was much lower than in plants with a single unpaired fragment. Intercrosses between plants with 14 + 2 s. f. or isolation of such plants gave progenies in which the average number of fragments was preserved but not increased. It is difficult to see, however, how the fragments can be maintained in populations of »Östgöta Gråråg», as the plants with 14 + 2 s. f. are slightly but significantly less fertile than plants without fragments (MÜNTZING, 1943). Plants with 4 extra fragments are still less fertile, and, further, the average number of fragments in the offspring of intercrosses between plants with 14 + 4 s. f. was found to be lower than 4. Thus, in »Östgöta Gråråg» the negative effects of fragment elimination at meiosis and fertility decrease in plants with fragments seem to be stronger than the positive effect of non-disjunction.

In »Vasa II» the situation is evidently quite different owing to the good pairing of the fragments at meiosis. As far as this material has yet been studied it showed a marked tendency to an increase in fragment number, and a similar situation seems to prevail in the material studied by LEWITSKY and his collaborators (1932). According to preliminary observations, the genetical effects of the fragments in »Vasa II» are similar to those of the fragments in »Östgöta Gråråg», i. e. a very slight influence on vigour and a somewhat stronger effect on fertility.

An interesting point in the present paper is the behaviour of the two types of iso-fragments derived from the standard fragment in »Östgöta Gråråg». The main result in this respect is the fact that the small iso-fragment has lost the ability of postmeiotic non-disjunction. The large iso-fragment, on the contrary, has retained this ability in full strength and was found to be just as capable of multiplication by means of non-disjunction as the standard fragment. On account of these results studies on the behaviour of the different fragment types in the pollen mitosis must be carried out.

The degree of fragment elimination during meiosis in plants with 2n = 14 + 1 iso-fragment was found to be about the same as in plants

with $2n = 14 + 1$ standard fragment. Probably, however, the rate of elimination of the small iso-fragment in such plants is somewhat higher than that of the large iso-fragments, the average percentages of gametes with fragments being 7,4 in plants with $2n = 14 + 1$ large iso-f. and 5,9 in plants with $2n = 14 + 1$ small iso-f.

The smallness of the small iso-fragment will probably prevent its regular pairing in plants with 2 or more fragments of this kind. For that reason alone the fragment in question would not be able to maintain itself in the populations. A still greater handicap in this respect is its inability to multiply by means of non-disjunction.

The large iso-fragment would certainly be more successful in the population. It is quite capable of non-disjunction and, according to preliminary observation, also capable of pairing in plants having two such fragments. This is also evident from the offspring of the two plants carrying two large iso-fragments and one or two standard fragments. Though these plants had also been exposed to the pollen of plants with $14 + 1$ large iso-f. (the proportion of male gametes with large iso-f. in this pollen being rather low) 50 per cent of the offspring were found to contain large iso-fragments (cf. p. 467). Hence, by intercrosses between plants with $14 + 2$ large iso-f. it should be easy to get a strain with a rather regular occurrence of large iso-fragments.

The whole problem of the origin and function of the extra fragment chromosomes (or *accessory* chromosomes as Dr. HÅKANSSON and I agreed to call them; cf. HÅKANSSON, 1945) is still rather obscure. In rye they only seem to have deleterious effects and, as suggested to me by my collegue Dr. ÖSTERGREN, they may possibly have a kind of »parasitic» existence. In such a case they are of no use to the plant but are preserved in the populations owing to their peculiar ability of non-disjunction and their relatively harmless effects. According to this view, the fragment chromosomes have succeeded in maintaining themselves despite the fact that selection in the populations will favour plants without fragments.

DARLINGTON and others assume that the accessory chromosomes, occurring in *Zea*, *Sorghum* and other genera, have important functions in the nucleic acid metabolism of the cell (cf. DARLINGTON and UPCOTT, 1941; DARLINGTON and THOMAS, 1941). This interesting possibility as well as the general properties of accessory chromosomes is discussed in detail in a recent paper by HÅKANSSON (1945). This review gives a good survey of the present state of things in this field of research. It also shows that much work remains to be done before the origin

and function of the peculiar accessory chromosomes can be accurately understood.

SUMMARY.

(1) A number of cross combinations in the rye variety »Östgöta Gråråg», involving plants with extra fragment chromosomes (»standard fragments»), gave the main result that the standard fragments are capable of an almost regular postmeiotic non-disjunction, leading to a doubling of the fragment number. This process is equally well developed in the ovules as in the pollen. The experimental results also strongly indicate that the process of non-disjunction is directed in such a way that the fragments generally pass to the male and female gametes. This is in accordance with HASEGAWA's observations on the behaviour of the fragments in the pollen mitosis.

(2) The transmission of small and large iso-fragments was studied in various cross combinations. These iso-fragments are derived from the short and long arms of the standard fragment in »Östgöta Gråråg». The large iso-fragment has the same ability of postmeiotic non-disjunction as the standard fragment, but the small iso-fragment is entirely devoid of this ability.

(3) Fragment chromosomes in the rye variety »Vasa II» were found to be of the same general type and to have the same ability of non-disjunction as the standard fragments in »Östgöta Gråråg» and the fragments occurring in Russian and Japanese populations. The fragment in »Vasa II», however, was found to be slightly but significantly larger than the fragment in »Östgöta Gråråg». It also showed a much higher degree of pairing and hence a higher average number of fragments in the offspring. The different degree of pairing is supposed to be due to genotypical control rather than to the slight size difference.

LITERATURE CITED.

1. DARLINGTON, C. D. 1933. The origin and behaviour of chiasmata. VIII. *Secale cereale* (n, 8). — Cytologia, Vol. 4, No. 4, pp. 444—452.
2. DARLINGTON, C. D. and THOMAS, P. T. 1941. Morbid mitosis and the activity of inert chromosomes in *Sorghum*. — Proc. of the Roy. Soc. of London, Vol. 130, pp. 127—150.
3. DARLINGTON, C. D. and UPCOTT, M. B. 1941. The activity of inert chromosomes in *Zea mays*. — Journ. of Genetics, Vol. 41, pp. 275—296.
4. HASEGAWA, N. 1934. A cytological study on 8-chromosome rye. — Cytologia, Vol. 6, pp. 68—77.

5. HÅKANSSON, A. 1945. Überzählige Chromosomen in einer Rasse von *Godetia nutans* HIORTH. — Botaniska Notiser 1945, H. 1, pp. 113—131.
6. LEWITSKY, G. A. 1931. The morphology of chromosomes. — Bull. Appl. Bot., Genet. a. Plant-Br., Vol. 27, pp. 19—174.
7. LEWITSKY, G. A., MELNIKOV, A. N. and TITOVA, N. N. 1932. The cytology of the offspring of the 16-chromosome rye. — Bull. Lab. Genet., Vol. 9, pp. 89—96.
8. MÜNTZING, A. 1943. Genetical effects of duplicated fragment chromosomes in rye. — Hereditas XXIX, pp. 91—112.
9. — 1944. Cytological studies of extra fragment chromosomes in rye. I. Iso-fragments produced by misdivision. — Hereditas XXX, pp. 231—248.
10. MÜNTZING, A. and PRAKKEN, R. 1941. Chromosomal aberrations in rye populations. — Hereditas XXVII, pp. 273—308.
11. STONE, L. H. A. and MATHER, K. 1932. The origin and behaviour of chiasmata. IV. Diploid and triploid *Hyacinthus*. — Cytologia, Vol. 4, No. 1, pp. 16—25.

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A CROSSING-OVER DECREASE CAUSED BY A NEW SEX-LINKED LETHAL

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IN 1927 MULLER opened up new ways in the experimental research of mutations by the discovery that the mutation frequency in *Drosophila melanogaster* could be increased by X-rays. Since that time a great many new ones and almost every mutation known before have been induced by X-ray treatment. Among these, the sex-linked lethals are the easiest to distinguish, thanks to the *ClB* method devised by MULLER. A great number of such lethals have been located and are described in »Drosophila Information Service», No. 9, 1938.

In the spring of 1944 a number of wild-type males were X-rayed at the Radiophysical Institute, Karolinska Sjukhuset, Stockholm. The dose was 4500 r. In the *X* chromosome of one of these males a lethal mutation was found. The purpose of our investigation was to locate the lethal, *lr*, and to find the reason why the crossing-over percentage was reduced in the whole *X* chromosome, a fact discovered during the course of the research.

The investigation has been performed at the Animal Breeding Institute, Wiad, Eldtomta. For help and advice in our work we are indebted to Professor GERT BONNIER.

Crossing-over frequency. — The F_1 Bar females, produced in the *ClB* cross, have been mated to males of the constitution *y ec ct v f*. The wild-type daughters from these matings have the constitution *y ec ct v f/lr*. When mated again to *y ec ct v f* males their offspring consisted of twice as many females as males, as the lethal had rendered half the number of males inviable.

New crosses were made, phenotypically normal females of the gene constitution *y ec ct v f/lr* being always mated to *y ec ct v f* males. During three generations the animals of 43 cultures were counted and classified, yielding a total of 5102 females and 3054 males. The distribution of these with reference to their characters is shown in Table 1. From these values the crossing-over percentage was calculated, both for all the animals together and for females and males separately, to ascertain whether there was any distinct deviation between them.

TABLE 1. *Offspring from the cross y ec ct v f/lr × y ec ct v f.*

Cr. o. in interval	O *y ec ct v f*	O +	I *y*	I *ec ct v f*	II *y ec*	II *ct v f*	III *y ec ct*	III *v f*	IV *y ec ct v f*	IV *f*	I, II *y ct v f*	I, II *ec*	I, III *y v f*
Females	2004	2110	40	48	76	103	30	32	321	292	1	1	—
Males	2249	2	1	43	—	130	24	6	374	1	2	—	1
Total.....	4253	2112	41	91	76	233	54	38	695	293	3	1	1

Cr. o. in interval	I, III *ec ct*	I, IV *y f*	I, IV *ec ct v*	II, III *y ec v f*	II, III *ct*	II, IV *y ec f*	II, IV *ct v*	III, IV *y ec ct f*	III, IV *v*	Total
Females	1	3	7	1	1	9	14	2	6	5102
Males	—	—	4	—	2	—	14	1	—	2854
Total.....	1	3	11	1	3	9	28	3	6	7956

TABLE 2. *Crossing-over values obtained from Table 1.*

		Non-cr. o.	I	II	III	IV	I, II	I, III	I, IV	II, III	II, IV	III, IV	Total
Females	Numbers	4114	101	206	73	654	2	1	10	2	23	8	5102
	%	80,64	1,98	4,04	1,43	12,82	0,04	0,02	0,20	0,04	0,45	0,16	
Males	Numbers	2251	51	148	34	394	2	1	4	2	14	1	2854
	%	78,87	1,79	5,19	1,19	13,81	0,07	0,04	0,14	0,07	0,49	0,04	
Total	Numbers	6365	152	354	107	1048	4	2	14	4	37	9	7956
	%	80,00	1,91	4,45	1,34	13,17	0,05	0,03	0,18	0,05	0,47	0,11	
Standard values			5,5	14,5	13,0	23,7							

Table 2 shows the result of these calculations. It appears that the difference between males and females is inconsiderable, and from now on we can calculate with the total values.

For comparison a number of crosses *y ec ct v f*/+ × *y ec ct v f*

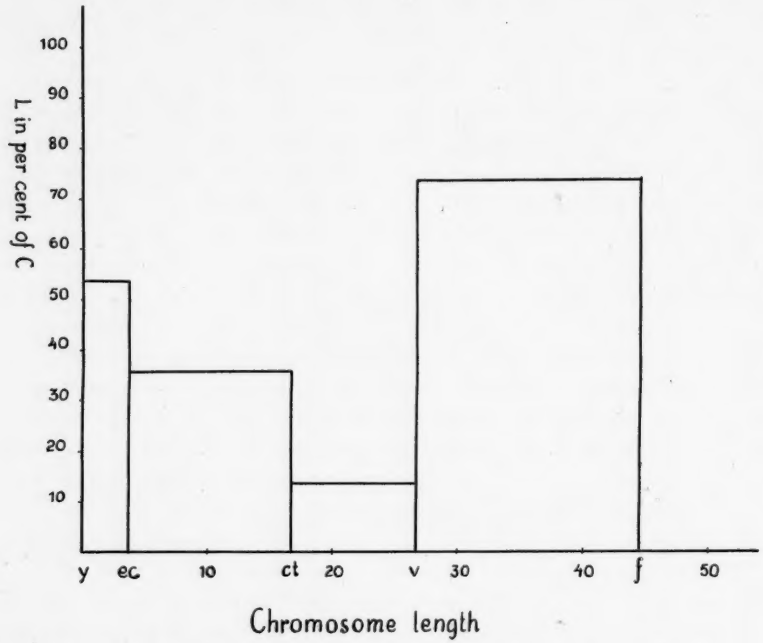

Fig. 1. The crossing-over decrease in the lethal cross. (L, lethal values; C, control values.)

TABLE 3. *Comparison between the values of single crossing-over for the control and for the lethal cross.*

	Interval			
	I *y—ec*	II *ec—ct*	III *ct—v*	IV *v—f*
Control cross (C)	3,6	12,9	9,7	18,4
Lethal cross (L)	1,9	4,5	1,3	13,2
L in per cent of C.....	52,8	34,9	13,4	71,7
χ^2	0,803	5,469	7,274	1,469
P	0,5—0,3	0,02—0,01	0,01—0,001	0,3—0,2

were performed. From these controls the animals of 10 cultures were counted, and gave 1498 females and 1357 males. If the values for the single crossing-over from the lethal cross are compared with those obtained from the control, we find that all the values from the lethal cross are inferior to those of the control (Table 3). The crossing-over reduction of the lethal cross is greatest in that interval where the lethal is situated, and it decreases in both directions (see below). The χ^2 values suggest an effect from some biological factor for at least two of the intervals.

To illustrate more clearly the crossing-over decrease the diagram in Fig. 1 is constructed. The abscissa shows the length of the chromosome, according to the values obtained from the control, and the loci of the genes in question have been inserted. On the ordinate the

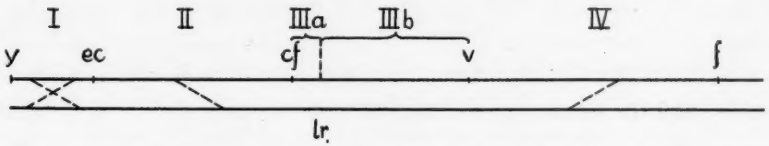

Fig. 2. Crossing-overs which lead to the coupling of the lethal with recessive genes.

crossing-over values of the lethal cross have been noted for the different intervals in per cent of those of the control.

Similarly, the values for the double crossing-over are considerably lower than those of the lethal cross, but comparisons are uncertain owing to the small numbers of animals.

Localization of the lethal. — The results of the crossing-over prove that the lethal must be situated somewhere between *ct* and *v*. In Fig. 2 we see that males which have received the gene constitution *y*, *y ec* and *f* after single crossing-over, or *y f* and *ec* after double crossing-over, must also have the lethal, if this is situated in Interval III. Such males are also lacking among the animals classified. Exceptions are three males, one with the genes *y ec*, one with *y* and one with *f*. To this class also belong the two wild-type males that have arisen in the crosses (see Table 1). As an explanation for the origin of these animals there are two possibilities: the gene may be a semilethal (cf. Discussion), or the animals may have lost the lethal in their *X* chromosome by double crossing-over. The origin can be explained as to the

+	animals	by	crossing-over	in	Interval		III a, III b;
yec	»	»	»	»	»	»	II, III a, III b;
y	»	»	»	»	»	»	I, III a, III b;
f	»	»	»	»	»	»	III a, III b, IV.

We tried to cross out these males with $\widehat{XX}$ females, but without success.

In order to localize the lethal, the total number of males having crossing-over in Interval III were counted. Their number was 34, and they could be divided into two groups: one with crossing-over to the left of the lethal within III a, and one with crossing-over to the right within III b (cf. Fig. 2).

Crossing-over in III a: 6 *v f* and 1 *y v f*. Total 7.
» » » III b: 24 *y ec ct*, 2 *ct* and 1 *y ec ct f*. Total 27.

The distance between *ct* and *v* was, according to the control, 9,8 units, and the length of the Interval III a was therefore $\frac{7 \times 9{,}8}{34} = 2{,}02$ units. The gene *ct* is situated at 20,0, and the locus of the lethal is 22,0.

Balancing the lethal. — The lethal was kept in stock by means of a balancing method indicated by MULLER (DIS, No. 6, 1936). Phenotypically normal females, *y ec ct v f*/*lr*, were mated to males with the constitution *dl—49, y Hw w lz*^{s}. *dl—49* is an inversion including 33 units. It extends from the point 11 to the point 44. *lz*^{s} homozygous females are sterile, whereas *lz*^{s} males are fertile. From F_1 were selected females not showing the character *y* and probably having the genotype *dl—49, y Hw w lz*^{s}/*lr* (if crossing-over had occurred in the *X* chromosome of the mothers, they might have been of a different kind, but in that case it would be detected in the following generations). They were again mated to *dl—49, y Hw w lz*^{s} males. All the sons were of the constitution *dl—49, y Hw w lz*^{s}, the females, on the other hand, were of two kinds: homozygous *dl—49, y Hw w lz*^{s} females and heterozygous *dl—49, y Hw w lz*^{s}/*lr* females. As only females of the latter genotype are fertile, the lethal is thus kept in stock.

Is the crossing-over decrease caused by the lethal? — The crossing-over decrease can be caused directly by the lethal, but it is also possible that it arises through influence exerted by some other part of the chromosome. To solve this problem we needed a chromosome where the part containing the lethal gene was exchanged, but which was

otherwise identical with the original, X-rayed chromosome. The two above-mentioned wild-type males had such *X* chromosomes. The outcrosses of these were not successful. With $\widehat{XX}$ females, however, one male only showing *v* gave sons that all had their *X* chromosome from the father. In this chromosome, crossing-over had occurred between *ct* and *v* and between *v* and *f*. The reason why the *v* male was crossed out, was also the possibility of proving a possible semilethal. If crossing-over had occurred between *lr* and *v*, the *X* chromosome of the

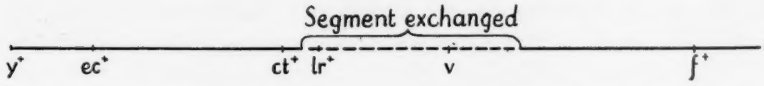

Fig. 3. The *X* chromosome used in the *v* experiment.

males must still contain *lr*. After the outcross with the $\widehat{XX}$ females we would expect only a few males. The result, however, did not indicate any lower vitality among the sons, and their *X* chromosome was therefore lacking in *lr*. Its appearance is shown in Fig. 3. As no *y ec ct f* female was to be had, we had to cross out these *v* males with totally recessive females — not a very good expedient, since the Intervals III and IV in this way could not be analysed separately. *v* females from F_1 were crossed to *y ec ct v f* males, and their offspring were classified.

TABLE 4. *Comparison between crossing-over values.*

	Interval		
	I	II	III + IV
Control cross	3,6	12,9	36,7
v cross	2,2	10,1	31,0
Lethal cross	1,9	4,5	14,5

A comparison of these crossing-over values with those of the control and the lethal cross is made in Table 4. As the Intervals III and IV in the experiment with the *v* male are unified, this segment has become so large that some double crossing-over must have occurred without being detected. The crossing-over percentage of these intervals is therefore too small. The values from this experiment are throughout slightly lower than those from the control, but considerably higher than the values from the lethal cross, particularly in the intervals where the decrease is most pronounced. Thus it is obvious that the low crossing-

over values are caused by the lethal itself and not by any other part of the chromosome.

Connection between crossing-over frequency and non-disjunction. — The results of the last-mentioned crosses show that the lethal has directly caused the low crossing-over percentage. As a possible explanation one might suppose the lethal to cause an incomplete pairing between the two *X* chromosomes. This fact would by itself result in a decrease of the crossing-over frequency, but would also lead to a non-disjunction, which in its turn would contribute to the decrease of the

TABLE 5. *Offspring from the cross y ec ct f/lr × B.*

Females						Males		Culture No.
B	+	*y ec ct v f*	*y ec ct v*	*ct v f*	*f*	Regular	*B*	
112	3					45	6	1
42					4	31	1	2
29						11		3
45						27	1	4
94	1				2	42	5	5
106	3					34	2	7
19						13		8
28	1					15		9
31						21		10
61						25		11
92	4	3	1	1		41	2	13
40		1				12	1	17
6						2	1	18
63	18					27	24	29
81	1					38	1	20
849	31	4	1	1	6	384	44	—

crossing-over values. Such a non-disjunction was impossible to dectect during the experiments hitherto performed, as we were working with gene combinations not marking the course of the chromosomes.

When balancing the lethal some animals were, however, hatched, giving support to this hypothesis, because after the cross *y ec ct v f/lr × dl—49, y Hw w lz*^{s} we obtained regular as well as patrocline sons. In this cross the *X* chromosome of the father was marked.

To get further evidence we made the cross *y ec ct v f/lr × B* (Table 5), according to which those daughters that have wild-type eyes are matrocline and the *B* sons are patrocline. As a result of a

combination of crossing-over and non-disjunction the daughters can show all or a few of the recessive characters. The totally recessive females have arisen after crossing-over between *f* and the centromere, *y ec ct v* females after crossing-over in Interval IV and *ct v f* and *f* females after double crossing-over, firstly between *f* and the centromere, secondly in Interval II and IV, respectively.

TABLE 6. *Percentage of non-disjunction animals in the cross y ec ct v f/lr × B. Corr.: the number of regular males doubled.*

	Females	Males	Males corr.	Total	Total corr.
Total	4,82	10,28	5,42	6,59	5,11
Except No. 19	3,08	5,31	2,75	3,79	2,91
No. 19	22,22	47,06		31,82	

The 15 cultures gave varying results. In four of them we obtained no non-disjunction animals. All the remaining cultures gave exceptionals, but one of them (No. 19) had a much higher percentage than the others. Table 6 shows the percentage of exceptionals, both for all the cultures together and for all except No. 19. An explanation of the result will be given further on.

Discussion. — Through X-ray treatment a lethal mutation has been induced, situated between *ct* and *v*, approximately 2,0 units from *ct*. The backcrosses also showed a crossing-over percentage which was too small. The crossing-over percentage of the whole distance *y—f* was only 20, corresponding to the 41,4 of the control. This marked decrease of the crossing-over percentage might depend on a chromosome mutation, perhaps an inversion, having arisen simultaneously with the lethal. The crossing-over values of the lethal — 4,5 % between *ec* and *ct* and 1,3% between *ct* and *v* — stand against those of the control — 12,9 % and 9,7 %. Both between *ec* and *ct* and between *ct* and *v* the crossing-over value is reduced by 8,4 units. This decrease would correspond to an inversion of 16,8 units. But with such an inversion, which would contain the lethal, it is difficult to explain the origin of the 6 *vf* males and the *y v f* male (Table 1). The former must have arisen after a three-double crossing-over in the interval between *ct* and *v* (9,7 units), the latter in the same way but with an additional crossing-over between *y* and *ec*. The crossing-over percentage is also abnormally low in the other parts of the chromosome, outside

of the possible inversion. Thus the crossing-over value is only 1,9 % between *y* and *ec* (3,6 % in the control) and 13,8 % between *v* and *f* (18,4 % in the control). Since an inversion only reduces the crossing-over value in the inverted part and does not influence the rest of the chromosome, an inversion cannot have caused the crossing-over decrease so obvious in all intervals.

In case of the X-ray mutation being a semilethal, the two wild-type males found might be non-crossovers, and the males with the gene constitution *y*, *y ec* and *f* (Table 1) might have arisen after single crossing-over. In such a case, however, a much greater number of males, which would have died rather young, would have been expected. Nothing of the kind has been observed, and, moreover, the experiment with the *v* male gives further proofs that no semilethality is present. After the outcross with $\widehat{XX}$ females, 48 sons arose, fully fertile and vital. The crossing-over values obtained from their offspring agree very closely with those of the control, but show great deviations as against those of the lethal cross.

Hence neither an inversion nor a semilethal can explain the phenomenon thoroughly. Then the lethal must have directly influenced the crossing-over decrease, and one would suppose a more or less suppressed synapsis in the meiosis to be the cause thereof. According to DARLINGTON (1937), a normal meiotic division presupposes that the chromosomes at chromatid formation are tied to each other by chiasmata, which guarantee a regular separation in the anaphase. If the pairing should be hindered by a possible repulsion, the chance of chiasmata forming must also be reduced, and the stronger the repulsion, the smaller the chances of pairing and the fewer the chiasmata formed. In our crosses, the interval in which the lethal is located also shows the greatest decrease of crossing-over, which, seen from a cytological point of view, is the result of a suppressed pairing in this interval. It may even happen that the pairing is completely suppressed, or in any case so slight that no chiasma is formed. In such a case the repulsion during the pachytene stage will separate the chromosomes, and they arrive in the metaphase as univalents. If the centromeres of the univalents should happen to lie on the same side of the equatorial plate, the two chromosomes will go together during the anaphase (STERN, 1929). When this primary non-disjunction concerns the *X* chromosomes, it gives rise to exceptions, matrocline daughters and patrocline sons. If a chromosome pairing, directly reduced by the lethal, *a priori* lowers the crossing-over frequency, this decrease will be further ac-

centuated by the presence of some, primary as well as secondary, non-disjunction animals. Since the saturation between two paired chromosomes is total, should three homologous chromosomes be present, only two of them can be simultaneously paired within a certain segment. If one does not accept the possibility of one bivalent and one univalent — which e. g. STERN considers improbable — the repulsion between the lethal and its allelomorph might be compensated by an attraction to the *Y* chromosome within this segment.

In the original backcrosses the wild-type females could be of different kinds: mostly there are ordinary diploid animals with *2X 2A*, besides these there are primary non-disjunction females with two maternal *X* chromosomes, and finally secondary non-disjunction females of two types: some with two maternal *X* chromosomes and one *Y* from the father, and some with *XY* from the mother and the other *X* chromosome from the father.

In the Bar experiment only one female has been used in every culture (except No. 19) without any knowledge of their chromosome constitution. In the cultures 3, 4, 8, 10 and 11, for instance, the mothers have in all probability been diploid. In culture 4 we thus found only one exceptional male, in No. 17 one exceptional male and one female. In those cultures where the daughters show recessive characters, however, the mothers cannot have been diploid, in agreement with the theory that in case of a primary non-disjunction the chromosomes are univalents in the metaphase of the first meiotic division. Since there are crossing-over animals present in these cultures, the mothers here must have been *XXY* females, they themselves having arisen after non-disjunction.

In culture 19, however, two wild-type females were mated to four *B* males, unfortunately, because this culture is very interesting. It is probable that one of the females in this culture was of the *XX* type. She then produced the 63 heterozygous *B* females and the 27 regular males (Table 5), and the sex-ratio also agrees rather well with the expected 2: 1. The *X* chromosomes of the other female must have been attached. The result of this attachment of course is wild-type daughters and *B* sons. This illegitimate attachment by crossing-over must have occurred latest in the grandmother, since these *B* males have fully motile sperms, and thus must be of *XY* type. This theory is further supported by the fact that the sex-ratio is approximately 1: 1, which is to be expected, as the lethal chromosome is bound to the females.

In Table 6 the percentage of non-disjunction appears to be twice

as high for males as for females. A correction, however, must be introduced here. The percentage of non-disjunction for the females is calculated for the total number of females, i.e. this value is the correct one, whereas the percentage values of males are too high, half the number of males having died, viz. those having received the *lr* chromosome from the mother. According to what has been said earlier this discussion does not hold for culture 19.

Of course, it would have been desirable to have each female examined; this would have given us information of their constitution and thus enabled us to calculate at what frequency the non-disjunction occurs.

SUMMARY.

(1) A sex-linked lethal (*lr*) induced by X-ray treatment has been localized to point 22,0.

(2) The lethal lowers the crossing-over frequency in the whole *X* chromosome.

(3) The crossing-over decrease is at its maximum in the interval of the lethal (between *ct* and *v*) and diminishes distally and proximally.

(4) The lethal causes a high primary non-disjunction.

LITERATURE CITED.

1. DARLINGTON, C. D. 1937. Recent advances in cytology. 2nd ed. — London.
2. Drosophila Information Service (DIS), No. 6, 1936 and No. 9, 1938.
3. MULLER, H. J. 1927. Artificial transmutation of the gene. — Science 66.
4. STERN, C. 1929. Über Reduktionstypen der Heterochromosomen von *Drosophila melanogaster*. — Biol. Zentralbl. 49: 718—735.

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ASCORBIC ACID IN ROSA HYBRIDS

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SVALÖF AND STOCKHOLM

IN a paper published by GUSTAFSSON (1944), concerning the mode of reproduction in *Rosa canina* forms, various morphological characters of hybrids were studied. In the course of experiments a strictly physiological property, viz. the formation of ascorbic acid in the ripe receptacles (hips), was also continually investigated. It is an established fact that different species in the genus *Rosa*, and even biotypes within a species, form widely different amounts of ascorbic acid (see, for instance, SCHRÖDERHEIM, 1941). Up to now, however, the behaviour of species hybrids has not been looked into.

The forms involved are those reported in a previous paper, viz. two *Rosa rugosa* biotypes, one cultivated in the Lund Botanical Garden, one at Svalöf; *R. rubiginosa*, two individuals from Lund and Svalöf; *R. canina* var. *Blondaeana*, Lund, and two highly different biotypes of *R. canina*, Svalöf.

Hips of the parents, as well as of the hybrids available, were analysed in 1941, 1942, 1943 and 1944. In the year 1944 the analyses were especially extensive, comprising up to four tests of each individual in order to work out exactly the average amount of ascorbic acid in fully ripe but not over-ripe hips. It was shown by SCHRÖDERHEIM (1941 and unpubl.), as well as by other scientists, that in certain species the ascorbic acid is rapidly destroyed after the ripening of the hips.

For the methods used the reader is referred to the cited paper by SCHRÖDERHEIM.

A COMPARISON BETWEEN PARENTS AND HYBRIDS.

The *R. rugosa* biotypes examined by SCHRÖDERHEIM have an average ascorbic acid content of 3 to 5 % (on dry matter of the flesh), varying between 2 % and 8 %. The two forms used in cross experiments fall nicely within the variation sphere. In 1944 they gave average values of 3,1 and 2,7 % respectively, the highest values being 3,6 and 3,0 %. Previous figures are rather similar.

R. rubiginosa, probably introduced into Sweden and rather uniform in appearance, shows a range from about 2—4,5 % (different in different years). The forms used in cross experiments (Lund, Svalöf) gave in 1944 an average content of 2,9 and 2,9 %. One individual, obtained from seeds of the Lund representative and growing in the experimental field at Svalöf, showed in 1944 an average value of 3,1 %, i. e. almost exactly the same as the mother.

R. canina var. *Blondaeana* has always given an extremely low figure, namely 0,7—1,1 % (1941), 0,6—0,9 % (1943) and 0,7—0,8 % (1944). A progeny plant, now cultivated at Svalöf, similarly produced 0,7—0,8 % in 1944. Very few of the *canina* types (s. l.) examined by SCHRÖDERHEIM had such a consistently low content. This result is the more remarkable, since the form in question has rather large and intensely red-coloured hips when ripe and, in addition, is one of the few *R. canina* microspecies containing 42 chromosomes. Two other hexaploids with a very low content of ascorbic acid are *R. Jundzillii* and *R. stylosa* var. *evanida*. The other *R. rubiginosa* and *canina* forms examined here possess the pentaploid number.

The two *R. canina* types (I and II) gave average values of 2,4 and 2,1 % in 1944. A progeny plant of *R. canina* II has a somewhat lower content, 1,8 %, but in 1943 this was 2,6 %. There is hardly any true difference, the more so as the mother and progeny look alike in morphological respects.

Between the species described here several different cross combinations were performed, unfortunately at such an early time that the importance of *Rosa* species as a source of ascorbic acid was not recognized. The results obtained from the F_1 hybrids are so interesting and promising, however, that we must reckon with a rapid increase of cross combination work in a near future. Kärnbolaget A.B., Stockholm, now has in its possession a great number of new hybrids as well as progeny plants from the F_1's reported here.

The *canina—rugosa* hybrids differ in behaviour. *R. canina* var. *Blondaeana* with its extremely low content of ascorbic acid gives an intermediary F_1 content. In 1944 eight different individuals possessed an average maximum value of 2,1 %, the mother having 0,8 % and the father 3,1 %. The variation between the different F_1 individuals is very slight. Whether there is any hereditary difference between them or not, is difficult to tell, the figures running rather similar. This is also reflected by the apparent homogeneity of the F_1 generation (cf. GUSTAFSSON, 1944, p. 407).

The crosses between *R. canina* I and *rugosa* and *R. canina* II × *rugosa* are more interesting from a practical point of view. In both instances there is a pronounced F_1 increase in ascorbic acid. Five plants were obtained in the first-mentioned series (1934—1), and all of them form a higher amount of acid than the parents. On an average they had in 1944 a maximum content of 3,7 % as against 2,4 % for the mother and 2,7 % for the father. All the F_1 tests carried out in different years show consistently the same results. Plant 4 runs distinctly higher in 1944 than its sister plants, with an average content of 4,1 %. Plants 3 and 5 lie considerably lower (3,5 and 3,6 %). Similarly in 1943 plants 1 and 2 lay higher than plant 5 (no other individuals were examined). Consequently it is probable that the F_1 individuals behave differently. This F_1 heterogeneity is accompanied by an obvious segregation in morphological characters (GUSTAFSSON, l. c., p. 410).

No more than two plants were obtained in the combination *R. canina* II × *rugosa*, one of them being monosomic and severely damaged by the hard winters of 1940—1942. The normal sister plant gave 4,3 % ascorbic acid in 1944, the mother having 2,1 % and the father 2,7 %. Thus the F_1 increase is considerable.

In the *canina—rugosa* crosses the F_1's are strong and rapid-growing with an intense flowering and fruiting. The nut formation is greatly decreased, however, so that the receptacles usually contain just a few developed nuts. In no respect do the F_1 plants look lethal or disharmonious. The difference in F_1 behaviour may be explained in a regular Mendelian fashion. In the *Blondaeana—rugosa* hybrids the *rugosa* genes for high production of ascorbic acid are thinned out, so to say, the F_1 consequently being intermediary. In the *canina* I and *canina* II hybrids the *rugosa* genes meet with genes from the female also giving a rather high production of ascorbic acid, and so an F_1 transgression is induced.

Some time ago the present authors pointed out another possibility (GUSTAFSSON and SCHRÖDERHEIM, 1944), namely that the sterility as to nut formation may be of importance. This possibility will be discussed in another connection (p. 494).

The *rubiginosa—rugosa* crosses give partially disharmonious F_1's. This is especially so in the combination of *R. rubiginosa* and *rugosa* from the Lund Botanical Garden. In this instance there occurs some form of diplontic sterility, the flowers falling off and hips being formed to a low extent. Nevertheless, compared with their parents, the F_1's possess approximately the same or a slightly lower content of ascorbic

acid. In 1944 two plants gave the average values of 3,0 and 2,8 % as against 2,9 % for *R. rubiginosa* and 3,1 % for *R. rugosa*.

The F_1's of *R. rubiginosa* and *rugosa* of the Svalöf provenience are in no way so abnormal, although the vegetative system appears over-developed in a certain sense. Flowering and fruit-setting is abundant. Especially is that so in some plants, for instance, plant 8. The mother and the father plants possessed average contents of 2,9 and

TABLE 1. *Nut content and ascorbic acid in R. canina II × rubiginosa.*

Plant No.	Nut content Average 1943, 1944	Maximum amount of ascorbic acid			
		Dry matter of the flesh		Absolute amount per hip	
		1943	1944	1943	1944
5	8,0 %	5,0 %	4,1 %	33 mg.	26 mg.
2	9,5 %	4,4 %	4,3 %	27 mg.	20 mg.
4	9,2 %	4,9 %	4,2 %	30 mg.	23 mg.
Average	8,9 %	4,8 %	4,2 %	30 mg.	23 mg.
3	10,0 %	4,1 %	3,5 %	24 mg.	17 mg.
1	10,7 %	4,1 %	4,0 %	31 mg.	22 mg.
6	14,4 %	4,2 %	3,3 %	26 mg.	18 mg.
Average	11,7 %	4,1 %	3,6 %	27 mg.	19 mg.
R. canina II	37,2 %	2,7 %	2,3 %	11 mg.	7 mg.
R. rubiginosa	36,0 %	3,4 %	2,9 %	9 mg.	10 mg.

TABLE 2. *Nut content and ascorbic acid in R. rubiginosa × canina II.*

Plant No.	Nut content		Maximum amount of ascorbic acid			
			Dry matter of the flesh		Absolute amount per hip	
	1943	1944	1943	1944	1943	1944
34	24,5 %	22,5 %	4,6 %	3,8 %	9 mg.	9 mg.
21	35,0 %	40,7 %	2,8 %	2,5 %	6 mg.	6 mg.
51	44,0 %	—	2,1 %	—	3 mg.	—
48	—	46,3 %	—	2,0 %	—	3 mg.
50	—	46,3 %	—	1,9 %	—	3 mg.
47	—	46,7 %	—	2,2 %	—	4 mg.
43	—	47,3 %	—	2,0 %	—	4 mg.
38	—	47,7 %	—	2,2 %	—	3 mg.
Average 48—38	—	46,9 %	—	2,1 %	—	3 mg.
R. rubiginosa	32,5 %	37,8 %	3,4 %	2,9 %	9 mg.	10 mg.
R. canina II	31,5 %	40,0 %	2,7 %	2,3 %	11 mg.	7 mg.

2,7 % (in 1944). Eight F_1 plants showed from 2,4 up to 3,0 %, and an average content of 2,7 %, i. e. approximately as the parents.

Especially interesting are the crosses *R. canina II* × *rubiginosa* and reciprocal. These hybrids have been described at some length in the paper by GUSTAFSSON (l. c.). The reciprocal two hybrid series are quite dissimilar in morphology, as well as habit of growth, winter-hardiness, and so on. Similarly there are pronounced differences in the amount of ascorbic acid. In 1944 the six individuals of *R. canina* II × *rubiginosa* had an average value of 3,9 %, seven shrubs of *R. rubiginosa* × *canina* II a value of 2,3 %. The distribution in different years is shown in Tables 1 and 2.

In no case and in no year did any plant of the hybrid series *R. canina* II × *rubiginosa* fall below a value of 3,2 %. Nine individuals examined of the reciprocal series *R. rubiginosa* × *canina* II show an average content from 1,9 to 2,8 % in the years 1941—1944, and only one individual had a considerably higher amount (varying from 3,7 % in 1944 to 5,1 % in 1941). This aberrant plant deviates strikingly as to morphology and fertility, as indicated in a previous paper. It, also, was monosomic, containing 34 chromosomes as against 35 in the sister plants examined.

Another feature of interest is that all the plants of *R. canina* II × *rubiginosa* show an increase in amount of ascorbic acid, as compared with the parents. *R. canina* II, growing at its original habitat, had from 1,8 to 2,3 % in 1944, growing in the experimental garden it had from 1,6 to 1,8 %. *R. rubiginosa* possessed from 2,6 to 2,9 %. In 1943 the figures were 2,4—2,7 % for the progeny plant of *R. canina* II and 3,3—3,4 % for *R. rubiginosa*. It is immediately evident that every hybrid plant surpasses the parents, in some cases by one or two per cent. Consequently, here as well as in the *canina—rugosa* series, there is a striking F_1 increase in the formation of ascorbic acid.

The reverse hybrid series, with the exception of the aberrant monosomic plant, in no instance surpassed *R. rubiginosa*, and in several cases fell below the other parent also (*R. canina* II). This is the more remarkable as these hybrids in no respect can be denoted as abnormal or disharmonious. Instead, they flower and fruit abundantly. Therefore other conditions must be at play.

R. rugosa is one of the best vitamin-producing species, not only owing to its rather high percentage of ascorbic acid but also to its large and heavy hips. It is therefore interesting to note that the *absolute* amount of ascorbic acid, measured per individual hip, is surpassed in

three F_1 individuals. *R. rugosa* (Svalöf) had in 1944 an absolute amount of 19—26 mg. per hip (four measurements), *R. canina* I 7—9 mg. (three measurements). Plant 2 of this hybrid series showed 34 and 38 mg. per hip, pl. 4 26 and 30 mg. and pl. 5 28, 30 and 31 mg.

But the cross *R. canina* II × *rubiginosa* is by far a more remarkable one. The parents have 5—7 and 8—10 mg. respectively (1944, three and four measurements). The six hybrid plants in no instance had a lower or an identical amount. Pl. 1 showed 20—22 mg., pl. 2 18—20 mg., pl. 3 16—17 mg., pl. 4 21—23 mg., pl. 5 23—26 mg., and pl. 6 15—18 mg., the number of measurements in each case being two or three. The 1943 values are even more striking, the hybrid vitamin amount increased by about three times.

Of the reciprocal series, five individuals in 1944 possessed lower amounts than both parents, viz. no more than 3—4 mg. per hip. Deviating here as elsewhere are plants Nos. 34 and 21, which have decreased fertility and about the same absolute content as the parents.

Previously it was shown (GUSTAFSSON, l. c.) that at least when different biotype compounds (»species») are involved, the *canina* roses give true hybrids. This was proved by differences in morphological characters, although the F_1's in some cases strikingly resembled the mother parent. As shown in this paper, the physiological property concerned in the formation of ascorbic acid also indicates the same. The individuals of *R. Blondaeana* × *rugosa*, *R. canina* I × *rugosa*, *R. canina* II × *rugosa*, *R. canina* II × *rubiginosa* and reciprocal always produce different amounts from those of the mothers involved.

HIP FERTILITY AND ASCORBIC ACID.

There are two possibilities to explain the F_1 increase of ascorbic acid. The immediate explanation is of course that the parents contain different genes for a high production and these are brought together in the hybrid. This would be the general combination scheme of *AAbb* × *aaBB* → *AaBb*. With a complete or almost complete dominance of *A* and *B* the scheme would bring out the required F_1 increase. This explanation may hold true in the case of *R. canina* I and *R. canina* II × *rugosa*, but it does not suffice in the crosses *R. canina* II × *rubiginosa* and reciprocal.

But also in the last-mentioned two series a genic explanation is possible. If we postulate that in *R. canina* II the genes for high production of ascorbic acid lie in the univalents and that in the case of

R. rubiginosa they lie in the pairing chromosomes, then *R. canina* II × *rubiginosa* will receive ascorbic acid genes from both parents, but *R. rubiginosa* × *canina* only from the mother. Consequently, crosses in the first direction will bring out an evident F_1 increase, crosses in the opposite direction a decrease. In view of the different constitution of the heterogametic systems such an explanation is not to be excluded.

In a recent note GUSTAFSSON and SCHRÖDERHEIM (1944) pointed out that *R. rubiginosa* × *canina* II, which generally decreases the amount of vitamin, is greatly fertile, producing a high number of nuts per hip, in contrast to *R. canina* II × *rubiginosa*, which increases ascorbic acid but is highly sterile (a few nuts per hip). An exception was *R. rubiginosa* × *canina* II pl. 34, which increases ascorbic acid but simultaneously decreases fertility. Consequently we were inclined to connect the formation of ascorbic acid and sterility in some way, though by no means disregarding the importance of specific genes or the general genotypic constitution.

The average nut content of *R. canina* II × *rubiginosa* (1943 and 1944) relative to the amount of ascorbic acid is as shown in Table 1. It immediately becomes evident that decreased fertility and ascorbic acid are connected, both as regards percentages and absolute amounts. The three plants having the lowest nut content also have the highest contents of ascorbic acid. The same thing appears after analysing the 1943 and 1944 data of *R. rubiginosa* × *canina* II. Plants 34 and 21 decrease the fertility as compared with the rest and simultaneously increase their content of ascorbic acid considerably.

In the joint paper mentioned we had some further evidence. Admittedly two sets of data referred to heterogeneous materials, involving different *Rosa canina* compounds (cases 1 and 2), but in one normal species (*R. rugosa*) the analysis of co-variance showed a rather satisfactory P-value (0,02).

In two articles HARRISON and JACKSON (1944) as well as MELVILLE (1944) denied the possibility of a negative correlation. HARRISON and JACKSON pointed out that a spontaneous hybrid of *R. dumetorum* and *mollis* had an ascorbic acid content lying just between that of the parents (941 mg. per 100 gm. flesh against 724 and 1420 mg. in the parents). This is a situation simulated by the hybrids of *R. Blondaeana* × *rugosa*. Here the parents lie widely different. It seems obvious to us that a hybrid genotype, poor in ability to form ascorbic acid, cannot improve by a possible sterility so as to surpass the best parent. The *dumetorum—mollis* hybrid was vegetatively very vigorous but

generatively weak, most flowers falling off before fruiting and the hips probably more or less deformed. That is also the case in some *R. rubiginosa—rugosa* hybrids.

MELVILLE (l. c.) finds a slightly negative correlation between nut content and ascorbic acid in *Rosa canina*. The highest vitamin content is associated with a relatively low fertility. Nevertheless, low ascorbic acid figures are coupled with the highest and the very lowest nut percentages.

In view of these objections we have reconsidered the data and gathered more material concerning the negative correlation in *R. rugosa* and the *R. canina* complex (s. l.).

In *R. rugosa* a negative correlation has been found to occur in different years (1939: $P < 0{,}001$, 1940: no significance, 1941: $0{,}05 < P < 0{,}02$, 1942: $P = 0{,}02$). After dividing the large material examined into three different groups comprising (1) »ripe», (2) »ripe to soft», (3) »soft to loose» hip collections, the negative correlation within each individual group will disappear, however, or even change in a more or less positive direction. The apparently negative correlation in *R. rugosa* no doubt depends on the flesh partly adhering to the nuts when the over-ripe hips were analysed. Simultaneously the amount of ascorbic acid of the flesh will decrease owing to the over-ripeness. This is illustrated by the following figures, taken from the 1941-material.

Condition	Number of analyses	Nut content	Ascorbic acid of the flesh
(1) Ripe	212	17,8 %	3,61 %
(2) Ripe-soft	219	19,7 %	3,54 %
(3) Soft-loose	166	20,2 %	3,40 %

The result of this reconsideration is that no correlation — either negative or positive — is to be found in *R. rugosa*.

Matters are different however in the *R. canina* complex, taken in its widest sense and comprising all the heterogamous species with their numerous microspecies. Here, an obvious and rather strong negative correlation prevails, which does not depend on any factors analysed in *R. rugosa*, as far as we can see. These results will be published by SCHRÖDERHEIM. Similarly a negative correlation is illustrated by the *R. canina* collections, examined in our joint paper of 1944.

The chief results of the present investigation are that hybrids between different *Rosa* types and species differ from their parents with

regard to ascorbic acid content. The examined reciprocal hybrid series differ greatly. In several cases the hybrids increase the amount of ascorbic acid even in the F_1 generation and simultaneously decrease their nut content, i. e. get rather sterile. For the elaboration of ascorbic acid, therefore, cross experiments will be of immediate value. Whether the F_1 increase is due to a favourable gene combination exclusively or to an induced sterility also, is left undecided for the present. Nevertheless, the very striking conditions *within* the reciprocal hybrid series of *R. canina* × *rubiginosa* argue in favour of the latter explanation. Further analyses of reciprocal hybrids in F_1 and F_2 will no doubt reveal the actual conditions.

LITERATURE CITED.

1. GUSTAFSSON, Å. 1944. The constitution of the *Rosa canina* complex. — Hereditas XXX.
2. GUSTAFSSON, Å. and SCHRÖDERHEIM, J. 1944. Ascorbic acid and hip fertility in *Rosa* species. — Nature 153.
3. HARRISON, J. W. H. and JACKSON, G. A. D. 1944. Ascorbic acid and hip fertility in *Rosa* species. — Nature 153.
4. MELVILLE, R. 1944. Ascorbic acid and hip fertility in *Rosa* species. — Nature 153.
5. SCHRÖDERHEIM, J. 1941. Untersuchungen über den Ascorbinsäuregehalt in Hagebutten. — Kgl. Fys. Sällsk. Handl. (Lund) 52.

ABSTRACTS — KURZE MITTEILUNGEN

G. ÖSTERGREN: Equilibrium of trivalents and the mechanism of chromosome movements.

A statistical analysis of the equilibrium position of trivalents and bivalents during meiosis in autotriploid material of the grass *Anthoxanthum aristatum* BOISS. ($2n = 3x = 15$) has revealed the following regularities.

(A) Each individual trivalent is displaced out of the metaphase plate towards that pole towards which it is turning its two-centromere side. (As is well known, trivalents nearly always have two centromeres directed towards one pole and one towards the other; so they may be said to have a two-centromere side and a one-centromere side.)

(B) There is a joint displacement of the whole metaphase plate, tri- as well as bi-valents, out of the equator of the spindle towards that pole towards which the majority of the centromeres of the cell are directed. In other words, if there are many trivalents having their two-centromere sides in the same direction, each individual one of them is more strongly displaced in that direction than if there had been only few of them directed in this way, and the bivalents are also displaced in the same direction, although less strongly than the trivalents.

The equilibrium position of trivalents strongly indicates that centromeres are *attracted* to spindle poles by *forces increasing in strength with an increasing distance between centromere and pole*, and that *each centromere is attracted only by that pole towards which it is turned*. Forces of this type can account for the movement to the metaphase plate during pro-metaphase (metakinesis), co- and auto-orientation of centromeres during metaphase, as well as the poleward movement during anaphase. There is no necessity to assume that the movement *to* the plate is caused by another mechanism than the movement *away* from it. The change in behaviour of chromosomes at the transition from metaphase to anaphase is simply due to the change from doubleness to singleness through the falling apart of daughter centromeres at mitosis and partner centromeres at meiosis. The double metaphase centromere is turned towards both poles and attracted by both, and the single anaphase centromere is turned towards and attracted by only one pole. The metaphase centromere behaves as the simple sum of two anaphase centromeres. (The observed equilibria are in contradiction to DARLINGTON's theory of balanced repulsions, which theory can also be shown to be wrong by mere theoretical considerations.)

The characteristic displacement of trivalents cannot be a specific characteristic of this grass species, as it was possible for J. A. BÖÖK to confirm my observations on his triploid material of the salamander *Triton taeniatus*. When we also consider how closely similar the course of mitosis is in all higher organisms, we may safely conclude that these observations are of general validity.

The possible nature of the forces mentioned above will be discussed in connection with a more detailed description of this investigation.

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J. A. BÖÖK: Equilibrium of trivalents at metaphase.

The equilibrium position of the V-trivalents at metaphase in autotriploid *Triton taeniatus* ($2n = 3x = 36$) has been analysed. The present investigation took place in connection with G. ÖSTERGREN's work on meiosis in autotriploid *Anthoxanthum aristatum*, which paper should be consulted by the reader.

The meiosis in triploid *Triton* has been described in a previous paper (BÖÖK, 1945). Attention is drawn here only to the behaviour of the trivalents. From 12 cells at the stage of full M.I. 20 V-trivalents were drawn by the aid of a camera lucida at a magnification of 10×90 without selection. The distance from the centromeres to the corresponding spindle pole (centrosome) was measured on the drawings in millimetres as indicated by the dotted lines in Fig. 1. The geometrical errors of this measuring method, which are of no importance, will be discussed in the more extensive description by ÖSTERGREN.

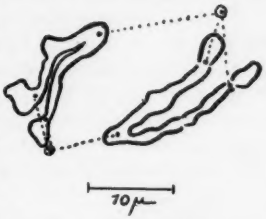

Fig. 1. Typical displacement of trivalents at M.I.

The V-trivalents, as is well known, have two centromeres directed towards one pole and one towards the other. We may conventionally call these »pair» and »single» centromeres respectively. The trivalents are found to be displaced out of the equator towards that pole towards which their pair centromeres are turned, as shown in Fig. 1. The analysis shows that this displacement is statistically significant (cf. Table 1).

TABLE 1.

a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
14,5	7,3	7,5	6,0	11,5	5,0	7,0	3,8
10,5	10,3	19,5	7,0	12,0	7,5	10,5	4,5
12,5	5,3	11,0	9,5	4,0	10,0	17,5	6,5
8,0	6,8	9,0	7,3	15,0	9,0	9,5	6,8
12,5	4,5	10,0	9,3	10,0	9,0	5,5	9,8

1 unit = 0,8 μ. — a = *single centromere—spindle pole* distance. — b = mean *pair centromeres—spindle pole* distance. — Diff. $a-b = x$. — $\Sigma x = +72{,}3$. — $\Sigma x^2 = 661{,}81$. — $\bar{x} = 3{,}615 \pm 1{,}039$. — $t = 3{,}52$. — $0{,}01 > p > 0{,}001$.

This characteristic behaviour of the trivalents indicates that the force in action between the spindle pole and the centromere is actually an *attraction*.

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Literature cited.

1. BÖÖK, J. A. 1945. Cytological studies in *Triton*. — Hereditas XXXI.
2. ÖSTERGREN, G. 1945. Equilibrium of trivalents and the mechanism of chromosome movements. — Hereditas XXXI.

HELGE JOHNSSON: Chromosome numbers of the progeny from the cross triploid × tetraploid *Populus tremula*.

Although *Populus tremula* is diploid, $2n = 38$, spontaneous triploids occasionally occur (JOHNSSON, 1940). Tetraploid plants occur in the progeny from the cross between triploids and diploids. The oldest tetraploid obtained in this way flowered in the spring of 1944.

In earlier papers the author has dealt with the chromosome numbers of the progeny from crosses between diploid and triploid aspens (1940), between triploid and triploid (1942), and between diploid and tetraploid (1945).

These investigations have shown that all chromosome numbers between $2x$ and $3x$ are realized from the diploid × triploid cross, although partly in reduced frequencies if the meiosis of the triploid is random; in addition $4x$ individuals appear in a frequency of 2,92 %. The chromosome numbers of the triploid × triploid progeny are more in accord with the probability computation, even if a certain selective elimination asserts itself. In the progeny after the cross diploid × tetraploid the chromosome numbers lie between 47 and 59.

Chromosome counts on 200 plants from the triploid × tetraploid cross have given the following results:

$2n$	50	51	52	53	54	55	56	57	58	59
No. of plants	0	0	0	0	1	1	3	3	1	8
Calculated ..	0,00	0,01	0,04	0,13	0,31	0,66	1,22	1,99	2,92	4,00

$2n$	60	61	62	63	64	65	66	67	68	69
No. of plants	7	7	17	16	24	15	16	13	14	9
Calculated ..	5,45	7,60	11,05	15,95	21,52	25,93	27,52	25,02	19,22	12,53

$2n$	70	71	72	73	74	75	76	77	78	79
No. of plants	15	3	3	5	6	3	0	0	0	0
Calculated ..	6,71	2,91	0,98	0,28	0,03	0,00	0	0	0	0

$2n$	80	81	82	83	84	85	86	87	88	89
No. of plants	0	0	0	1	4	1	0	0	0	0
Calculated ..	0	0	0	0	0	0,04	0,04	0,11	0,18	0,18

$2n$	90	91	92	93	94	95	96	97	98	102
No. of plants	0	0	0	0	0	2	1	0	0	1
Calculated ..	0,11	0,11	0,51	0,69	0,80	2,70	0,29	0,07	0	0

The calculated values have been obtained under the assumption that the meiosis of the triploid is random and with reference to the fact that 2,92 % unreduced gametes are formed. Furthermore, the gametes of the tetraploid have been assumed to have those chromosome numbers and to occur in those frequencies that have appeared in the cross diploid × tetraploid. The chromosome numbers observed agree rather well with the calculated ones even if certain deviations make their appearance. The chromosome numbers between 64 and 69 are therefore rather noticeably underrepresented, while the

numbers 72—75 (near $4x$) are supernumerary. The individuals between 83 and 102, which must take their origin from unreduced triploid gametes, represent 5 % of the total number, compared with the 2,92 % given for the diploid × triploid cross. There also occurred the numbers 83 and 84, which should not be realized for probability reasons. These deviations can be accounted for in different ways and scarcely take away the impression that the assumptions made are correct on the whole.

Further studies of this progeny are in progress.

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Literature cited.

1. JOHNSSON, H. 1940. Cytological studies of diploid and triploid *Populus tremula* and of crosses between them. — Hereditas XXVI.
2. — 1942. Cytological studies of triploid progenies of *Populus tremula*. — Hereditas XXVIII.
3. — 1945. The triploid progeny of the cross diploid × tetraploid *Populus tremula*. — Hereditas XXXI.

ESKO SUOMALAINEN: Vermutete Triploidie bei der parthenogenetischen Gewächshausschabe, *Pycnoscelus surinamensis* L. (Blattaria).

Die ursprüngliche Heimat der Gewächshausschabe, *Pycnoscelus surinamensis* L., liegt in den Tropen, wo die Art zwischen den beiden Wendekreisen fast überall anzutreffen ist (näheres bei ROESER, 1940). Dort werden von dieser Schabe sowohl Weibchen als auch Männchen gefunden. Aus den Tropen ist die Art nach Europa und Nordamerika wahrscheinlich mit Pflanzensendungen eingeschleppt worden. In den letztgenannten Ländern lebt sie in Gewächshäusern und ist oft als ein schwerer Schädling bekannt geworden. Hier sind von *Pycn. surinamensis* niemals Männchen, sondern stets nur Weibchen gefunden worden. Diese vermehren sich parthenogenetisch. Weil von *Pycn. surinamensis* in den Tropen beide Geschlechter, in den Ländern mit gemässigtem Klima dagegen nur sich ausschliesslich parthenogenetisch vermehrende Weibchen auftreten, repräsentiert die Art einen typischen Fall von geographischer Parthenogenese.

Von der Gewächshausschabe findet man wenigstens in Mitteleuropa zwei morphologisch verschiedene Rassen. ROESER (op. c.) bezeichnet diese beiden Rassen als »*major*» und »*minor*». Die Rasse *major* ist grösser und zeigt dunkelbraune bis schwarze Flügelfärbung, die Rasse *minor* ist kleiner und hellbraun. Z. B. in den Gewächshäusern des Botanischen Gartens in Berlin-Dahlem mit einer ziemlich konstanten Erdtemperatur von 17—21° C war ausschliesslich die Rasse *major* zu finden, während im Krokodilhaus des Zoo in Berlin mit etwa 20—25° C Erdtemperatur die Rasse *minor* vor allem zu Hause war. Ob die Rasse *minor* im allgemeinen wirklich eine höhere Temperatur fordert, ist jedoch unsicher. Z. B. im Krokodilhaus lebt ausserdem auch die Rasse *major*.

Es ist festgestellt worden (vgl. VANDEL, 1928; SUOMALAINEN, 1940;

SEILER, 1942, 1943; u. a.), dass die geographische Parthenogenese auf der Polyploidie der parthenogenetischen Rasse beruht. Darum erhebt sich die Frage, ob auch bei *Pycnoscelus surinamensis* die in den Ländern mit gemässigtem Klima vorkommende parthenogenetische Rasse polyploid ist. Auch verdiente untersucht zu werden, ob die beiden erwähnten *Pycn. surinamensis*-Rassen vielleicht verschiedene Polyploidiestufen repräsentieren.

Sowohl die Rasse *major* als die Rasse *minor* haben in ihren somatischen Zellen deutlich 36 Chromosomen (Fig. 1—2). Diese Zahl wurde bei beiden Rassen in manchen mitotischen Metaphasen an Follikelzellen des Ovars konstatiert. Die Chromosomen sind nicht gleich gross, die Grössenunterschiede sind aber so kontinuierlich und klein, dass eine genaue Analyse der einzelnen Chromosomentypen nicht möglich ist. Auch die X-Chromosomen kann man in den Mitosen nicht erkennen. Die meisten Chromosomen sind V-förmig und

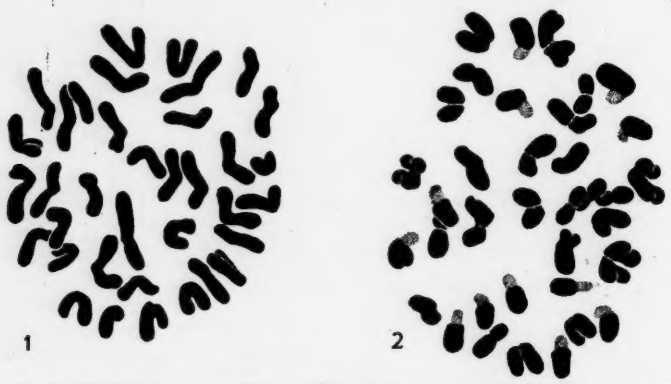

Fig. 1—2. Follikelmitosen von *Pycnoscelus surinamensis* mit 36 Chromosomen. — 1. Die Rasse *minor*. — 2. Die Rasse *major*. — 3000 ×.

scheinen einen medianen oder submedianen Spindelansatz zu haben. — Von den Reifungsteilungen habe ich keine Präparate erhalten können, obwohl ich dazu recht zahlreiche Weibchen präpariert habe. Die Weibchen gebären lebende Junge, deren früheste Embryonalentwicklung sich im Brutsack, also innerhalb des Körpers der Mutter, vollzieht (vgl. ROESER, op. c.). Die Eier durchlaufen vermutlich die Reifungsteilungen (oder die Reifungsteilung), gerade wenn sie aus den Ovariolen in den Brutsack gelangt sind. Diesen Moment kann man äusserlich an den Tieren nicht erkennen; darum ist es recht schwierig, die richtigen Stadien zu finden. Die Frage von der Zahl der Reifungsteilungen bei *Pycn. surinamensis* bleibt demgemäss offen.

Die bisexuelle Rasse von *Pycnoscelus surinamensis* ist zytologisch nicht untersucht worden. Unter jetzigen Umständen ist es unmöglich, von dieser Rasse Material zu bekommen. MORSE (1909) hat jedoch bei einer naheverwandten bisexuellen Schabenart, *Leucophaea maderae* F., die diploide Chromosomenzahl 24 konstatiert. (*Leucophaea maderae* ist allerdings von *Pycn. surinamensis* morphologisch verschieden und bedeutend grösser als diese).

Demnach scheint es wahrscheinlich, dass die parthenogenetische *Pycn. surinamensis*-Rasse in Europa triploid ist. — Auch MATTHEY (1942) hat *Pycnoscelus surinamensis* zytologisch untersucht und in Follikelzellen des Ovars 40 Chromosomen gefunden. Auch er schliesst aus seinen eigenen Untersuchungen bei *Pycnoscelus* und denjenigen von MORSE bei *Leucophaea*, dass die parthenogenetische Rasse von *Pycnoscelus surinamensis* polyploid ist.

Bemerkenswert ist, dass manche Chromosomen in den untersuchten mitotischen Metaphasen bei *Pycnoscelus surinamensis* aus zwei, sich deutlich verschieden färbenden Teilen bestehen (vgl. Fig. 2). Manche Chromosomen haben nämlich einen Endabschnitt, der schmäler als das übrige Chromosom ist und sich weniger intensiv färbt. Die in der Fig. 2 abgebildete Platte zeigt mindestens 12 Chromosomen mit einem deutlich helleren »Schwanz». Die exakte Bestimmung der Anzahl solcher Chromosomen ist schwierig, weil sich die Endabschnitte selbst in derselben Platte ein wenig verschieden stark färben. In einigen Platten sind sie sogar gleich dunkel gefärbt wie die übrigen Chromosomenteile, so dass man sie nicht erkennen kann (Fig. 1). In den Prophasen konnte ich diese helleren Endabschnitte nicht feststellen. Es ist offenbar, dass wir es hier mit einer Heterochromasie zu tun haben. — Es möge erwähnt werden, dass ich (unveröffentlicht) bei der Küchenschabe, *Phyllodromia germanica* L., im X-Chromosom einen helleren heterochromatischen »Schwanz» festgestellt habe, der sich jedoch nicht nur in der Metaphase, sondern auch während der anderen Phasen der Reifungsteilungen weniger intensiv färbt.

Diese Arbeit wurde zum grössten Teil im Kaiser Wilhelm-Institut für Biologie, Abteilung H. BAUER, durchgeführt. Das Untersuchungsmaterial stammt aus Berlin (die Rasse *major* aus dem Botanischen Garten in Dahlem, die Rasse *minor* aus dem Krokodilhaus des Zoo). Zur Fixierung und Färbung der Präparate ist Karminessigsäure verwendet worden [Quetschpräparate nach dem bei BAUER und TIMOFÉEFF-RESSOVSKY (1939) angegebenen Verfahren]. Sie wurden dann mit dem Gemisch von BOUIN-ALLEN nachfixiert und mit fuchsinschwefliger Säure nach FEULGEN nachgefärbt.

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Zitierte Literatur.

1. BAUER, H. und TIMOFÉEFF-RESSOVSKY, N. W. 1939. Vererbung und Vererbungscytologie von *Drosophila* im Schulversuch. — Der Biologe 8, pp. 324—335.
2. MATTHEY, R. 1942. Contribution à l'étude cytologique de la parthénogénèse chez les Orthoptères: *Pycnoscelus surinamensis* L. (Blatt. Panchloridae). — Verh. Schweiz. naturforsch. Ges. 122, p. 135.
3. MORSE, M. 1909. The nuclear components of the sex cells of four species of cockroaches. — Arch. f. Zellforsch. 3, pp. 483—520.
4. ROESER, G. 1940. Zur Kenntnis der Lebensweise der Gewächshausschabe *Pycnoscelus surinamensis* L. — Gartenbauwissensch. 15, pp. 184—225.
5. SEILER, J. 1942. Resultate aus der Kreuzung parthenogenetischer und zweigeschlechtlicher Schmetterlinge. — Arch. d. Julius Klaus-Stiftung 17, pp. 513—528.
6. — 1943. Über den Ursprung der Parthenogenese und Polyploidie bei Schmetterlingen. — Arch. d. Julius Klaus-Stiftung 18, pp. 691—699.

7. SUOMALAINEN, E. 1940. Beiträge zur Zytologie der parthenogenetischen Insekten. I. Coleoptera. — Annal. Acad. Scient. Fenn., Ser. A, 54, N:o 7, pp. 1—144.

8. VANDEL, A. 1928. La parthénogénèse géographique. Contribution à l'étude biologique et cytologique de la parthénogénèse naturelle. — Bull. Biol. France et Belgique 62, pp. 164—281.

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